AWARDS
AND
APPLICATIONS APPROVED FOR FUNDING

2007

CALIFORNIA INSTITUTE FOR

CIRM

REGENERATIVE MEDICINE

CIRM AWARDS AND APPLICATIONS APPROVED FOR FUNDING

2007 Report

About CIRM

Governed by the Independent Citizens Oversight Committee (ICOC), the California Institute for Regenerative Medicine (CIRM) was established in 2004 with the passage of Proposition 71, the California Stem Cell Research and Cures Initiative. The statewide ballot measure, which provided $3 billion in funding for stem cell research at California universities and research institutions, was approved by California voters, and called for the establishment of an entity to make grants and provide loans for stem cell research, research facilities, and other vital research opportunities. The CIRM is the largest source of funding for human embryonic stem cell research in the world. To date, grants totaling more than $208.5 million have been approved by the ICOC. For more information, please contact us at info@cirm.ca.gov or visit our website at www.cirm.ca.gov.

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EXECUTIVE SUMMARY

In November 2004, California voters passed Proposition 71 and embarked on a ground-breaking program to support stem cell research. Passage of Proposition 71, now known as the California Research and Cures Bond Act of 2004 (the Act), authorizes the use of $3 billion of General Obligation bonds over 10 years to fund pioneering research on human pluripotent stem and progenitor cells with the goal of developing treatments and cures to relieve human suffering. The California Institute for Regenerative Medicine (CIRM) is a state agency established by the Act to implement this mission under the guidance and oversight of a 29 member Independent Citizens Oversight Committee (ICOC).

In accordance with the mandate of the citizens of California, the ICOC determined that the mission of CIRM is:

> ***To support and advance stem cell research and regenerative medicine under the highest ethical and medical standards for the discovery and development of cures, therapies, diagnostics and research technologies to relieve human suffering from chronic disease and injury.***

To advance this mission, CIRM prepared a Scientific Strategic Plan which provides a blueprint for how the funds will be expended to provide maximal scientific and medical benefit. Although this document is intended to guide the activities of CIRM for the next 10 years, it is also intended to be a "living" document with the flexibility necessary to enable the institute to adapt to the changing scientific landscape.

Noting that the restrictions on federal funding have severely limited research on human embryonic stem cells, the strategic plan identified a critical need for scientists trained in this field. One of the first priorities in the plan is to support training in the stem cell field; another is to "jump-start" the use of human embryonic stem cells in research. Both would contribute to laying a foundation for the basic and discovery research needed for the discipline to grow.

In May 2005, CIRM issued a Request For Applications (RFA) to accelerate the training of young stem cell scientists in California. The goal of RFA 05-01 was to establish a CIRM Training Program to prepare exceptional pre-doctoral, post-doctoral and clinical fellows for careers in stem cell research through programs at institutions with strengths in areas of basic biology and/or clinical research. Eligibility was restricted to academic and not-for-profit institutions within the state; each institution was allowed to submit a single application. Of the 26 applications received, 16 were approved for funding by the ICOC in September 2005 at a total cost of $37.5 million. This program currently supports approximately 160 CIRM Scholars across the state.

CIRM's first initiative in support of basic and translational research was launched in 2006 to "jump-start" innovative new projects using human embryonic stem cells. This initiative has three goals: to attract new investigators and innovative ideas into the field, to support mature, ongoing studies by scientists with a record of accomplishment in this area, and to create dedicated laboratory space and training for the culture of human embryonic stem cells. CIRM issued three RFAs in support of these three goals: the Leon J. Thal SEED (Scientific Excellence

through Exploration and Development) Grant Program; the Comprehensive Research Grant Program; and the Program for Shared Research Laboratory Grants and Stem Cell Techniques Courses. The last RFA provided funding for space and equipment that is free of federal funding, and was the first opportunity for CIRM to address the hardships imposed by federal policies.

The Leon J. Thal SEED Grant Program (RFA 06-01) was named in memory of Dr. Leon J. Thal, a distinguished scientist and clinician specializing in research on Alzheimer's disease, who served on the ICOC from its inception until his untimely death in 2007. The goals of this program are to support ground-breaking concepts and approaches in research and to attract talented investigators to direct their focus on human embryonic stem cell research. These SEED grants offered investigators the opportunity to conduct pilot studies that may yield critical preliminary data or proof-of-principle results that could later be extended to full scale investigations. The program yielded 231 applications from principal investigators at academic and not-for-profit research institutions in California. The ICOC met in early 2007 and approved 74 proposals for funding at a total cost of $45 million.

The Comprehensive Research Grants Program (RFA 06-02) provides longer-term support for mature, ongoing studies on human embryonic stem cells by established scientists with a record of accomplishment in this field, and enables these investigators to expand their programs and to undertake promising new projects. Of the 70 applications received by CIRM, 29 proposals were approved for funding by the ICOC at a total cost of $74.6 million.

The main objective of the Shared Research Laboratory Grant Program (RFA 07-01) is to create dedicated laboratory space that is free of federal funding for the culture of human embryonic stem cells, including lines which fall outside the federal guidelines. With CIRM-funded space and equipment, these core laboratories provide an environment for the unrestricted conduct of research on these stem cells, and are intended to be shared by multiple investigators and multiple institutions. Another objective is to offer Stem Cell Techniques Courses within the CIRM-funded Shared Research Laboratories. These courses will provide hands-on training on derivation, growth and maintenance of human embryonic stem cells for scientists and technical staff in the state. In response to this RFA, 22 applications were submitted and 17 approved by the ICOC for funding in June 2007. Nine applications proposed the optional Stem Cell Techniques Course with 6 approved for funding. The total cost of this program is anticipated to be $50 million.

All funding decisions are made by the ICOC. Applications approved for funding then undergo administrative review by CIRM to ensure compliance with CIRM policies and regulations. An approved application is funded only if it fulfills all CIRM requirements.

This publication contains applications that have been approved for funding by the ICOC and have cleared an administrative review for eligibility.

CHAPTER 1

CIRM TRAINING GRANT PROGRAM

The CIRM Training Grant Program was developed to train a cadre of basic and clinical scientists who will contribute to the expansion of stem cell research in California. The growth of stem cell research in California will require the scientific training of individuals at all levels to provide a continuing supply of well-trained scientists with the knowledge and skill to conduct research in the field and eventually to lead effective research programs.

In April of 2006, CIRM awarded three-year training grants to 16 California academic or not-for-profit research institutions to foster training of pre-doctoral students, post-doctoral and clinical fellows. Each grantee institution offered unique strengths and areas of specialization in their stem cell research programs. Accordingly, the level of training offered by each institution differs. Three types of training programs were available to accommodate the varied institutional capabilities.

Type I - Comprehensive training programs: Comprehensive programs offer training at all three educational levels: pre-doctoral, post-doctoral and clinical. Each institutional grant may support up to 16 trainees, with a total (direct and indirect) cost/budget of up to $1.25 M per year. The apportionment of trainees among the different levels of education is determined by the institution. Type I grantees include universities with medical schools that have large research programs in stem cell research and well-established programs of graduate training.

Type II - Intermediate training programs: Intermediate programs offer training at two of the three levels of education (e.g., pre-doctoral and post-doctoral; post-doctoral and clinical; or pre-doctoral and clinical). Each award may support up to 10 trainees, with a total (direct and indirect costs) budget of up to $800,000 per year. Type II grantees include institutions that may have less extensive stem cell research programs, but with strong training opportunities.

Type III - Specialized training programs: Specialized programs offer training at one or two levels of education. Each grant may support up to 6 trainees, with a total (direct and indirect costs) budget of no more than $500,000 per year. Grantees include institutions with relatively small but developing stem cell research programs.

All training programs offered one or more classes in stem cell biology and its application to health and disease, as well as a course in the social, legal and ethical implications of stem cell research. Moreover, all programs offered opportunities for laboratory work under the direction of a mentor in stem cell biology or clinical training that is closely related to stem cell research. During the first year, approximately 160 trainee appointments were made under the CIRM Training Program. These trainees, the CIRM Scholars, are currently engaged in research training in a variety of fields including developmental biology, cell biology, neurobiology, molecular biology, cardiology, bioengineering, ethics and law. The selection of CIRM Scholars has become quite competitive among the different institutional programs, which suggests that our goal to educate and prepare exceptional trainees for careers in stem cell research is being realized through this initiative.

CIRM TRAINING GRANT AWARDS

Burnham Institute CIRM Stem Cell Training Grant
Burnham Institute for Medical Research
P.D.: Mark Mercola, Ph.D.
$1,384,005 for 3 years
Year 1:6 Post-doctoral fellows

Training in Stem Cell Biology at Caltech
California Institute of Technology
P.D.: Paul Patterson, Ph.D.
$2,071,822 for 3 years
Year 1:10 Post-doctoral fellows

Stem Cell Training Grant
Childrens Hospital Los Angeles
P.D.: Donald Kohn, M.D.
$2,352,018 for 3 years
Year 1:7 Post-doctoral fellows
3 Clinical fellows

Gladstone CIRM Scholar Program
The J. David Gladstone Institutes
P.D.: Robert Mahley, M.D., Ph.D
$2,397,239 for 3 years
Year 1:7 Post-doctoral fellows
3 Clinical fellows

Training in the Biology of Human Embryonic Stem Cells and Emerging Technologies
The Salk Institute for Biological Studies
P.D.: Juan Izpisua Belmonte, Ph.D.
$1,443,030 for 3 years
Year 1:6 Post-doctoral fellows

Training Stem Cell Researchers at the Chemistry-Biology Interface
Scripps Research Institute
P.D.: Peter Schultz, Ph.D.
$1,051,380 for 3 years
Year 1:3 Pre-doctoral fellows
3 Post-doctoral fellows

Stanford CIRM Training Program
Stanford University
P.D.: Michael Longaker, M.D., M.B.A.
$3,708,301 for 3 years
Year 1:6 Pre-doctoral fellows
5 Post-doctoral fellows
5 Clinical fellows

Human Stem Cell Training at UC Berkeley and Childrens Hospital at Oakland
University of California, Berkeley
P.D.: Randy Schekman, Ph.D.
$2,447,970 for 3 years
Year 1:6 Pre-doctoral fellows
4 Post-doctoral fellows
2 Clinical fellow

UC Davis Stem Cell Training Program
University of California, Davis
P.D.: Fredrick Meyers, M.D.
$2,688,246 for 3 years
Year 1:4 Pre-doctoral fellows
4 Post-doctoral fellows
4 Clinical Fellows

Stem Cell Research Training Grant
University of California, Irvine
P.D.: Peter Bryant, Ph.D.
$2,093,099 for 3 years
Year 1:8 Pre-doctoral fellows
4 Post-doctoral fellows

CIRM Comprehensive Training Program
University of California, Los Angeles
P.D.: Owen Witte, M.D.
$3,695,407 for 3 years
Year 1:5 Pre-doctorals fellows
5 Post-doctorals fellows
6 Clinical fellows

Interdisciplinary Stem Cell Training Program at UCSD
University of California, San Diego
P.D.: Lawrence Goldstein, Ph.D.
$3,609,620 for 3 years
Year 1:6 Pre-doctoral fellows
4 Post-doctoral fellows
6 Clinical fellows

Training Program in Stem Cell Research at UCSF
University of California, San Francisco
P.D.: Susan J. Fisher, Ph.D.
$3,515,379 for 3 years
Year 1:6 Pre-doctoral fellows
6 Post-doctoral fellows
4 Clinical fellows

UCSB Stem Cell Biology Training Program
University of California, Santa Barbara
P.D.: Dennis Clegg, Ph.D.
$1,218,241 for 3 years
Year 1:2 Pre-doctoral fellows
4 Post-doctoral fellows

Training Program in Systems Biology of Stem Cells
University of California, Santa Cruz
P.D.: David Haussler, Ph.D.
$1,132,201 for 3 years
Year 1:3 Pre-doctoral fellows
3 Post-doctoral fellows

CIRM Stem Cell Biology Training Grant
University of Southern California
PD: Robert Maxon Ph.D.
$2,703,942 for 3 years
Year 1:5 Pre-doctoral fellows
2 Post-doctoral fellows
2 Clinical fellow

ABSTRACTS

[Provided by grantee]

Burnham Institute CIRM Stem Cell Training Grant

Burnham Institute for Medical Research Program Director: Mark Mercola, Ph.D.

Burnham Institute for Medical Research proposes a CIRM type II program to train pre-doctoral PhD students and post-doctoral scientists. Currently, Burnham Institute faculty direct a large stem cell research and teaching enterprise that comprises over 100 biologists, chemists, engineers and clinicians with extensive expertise in stem cell biology and in allied disciplines dedicated to stem cell-based therapies for cardiovascular, neurodegenerative, hematopoietic and metabolic disorders. Additionally, the Institute has made substantial technology, recruitment and infrastructure investments as part of its commitment to stem cell biology. Our training curriculum will incorporate 1) an intensive hESC training course that has been run for two years and offers practical, hands-on instruction, 2) additional courses in stem cell, development, animal models of disease, bioinformatics and chemical biology, 3) training in ethical and legal implications of stem cells, and 4) laboratory research. Courses will be open to other CIRM program trainees in the La Jolla area and students will benefit from our inter-institutional research and training collaborations. PhD degrees will be granted through our existing training partnership with UCSD and through an independent Burnham PhD program.

Training in Stem Cell Biology at Caltech

California Institute of Technology Program Director: Paul Patterson, Ph.D.

The Caltech Stem Cell Biology Training Program will educate postdoctoral scholars in stem cell biology, its various potential medical applications, as well as the social, ethical and legal issues in this field. In addition to our present stem cell course offerings, we have organized a new bioethics course that emphasizes issues raised by stem cell research and applications. We have also initiated a collaboration with the Keck School of Medicine/University of Southern California (USC) and the Childrens Hospital of Los Angeles (CHLA) to offer a new tri-campus lecture course in stem cell biology.

The major strengths of a CIRM training program at Caltech are the extremely high quality of the trainee population, the strength and cross-disciplinary nature of research offerings, the research facilities, and the available and new courses. Relevant areas of current research at Caltech include embryonic and adult stem cell plasticity, stem cells and cancer, embryonic development, imaging technology, tissue engineering and macromolecular fabrication, computational biology, nanoscale biology and chemistry, and the basic science of hematopoietic, muscle, endothelial and neural stem cells. The cells and organisms being studied in this context include yeast, C. Elegans, Drosophila, Xenopus, zebrafish, chick, rodents and humans. The new, collaborative part of this training program utilizes the expertise at Keck/USC and CHLA in the areas of human embryonic stem cell growth and differentiation, cutting edge gene transfer technology application in the clinic, stem cell research in a variety of organs, as well as medical ethics. Together, these institutions can provide a broad, in depth curriculum for trainees. This collaboration also offers the opportunity and stimulus for basic scientists to become familiar with related clinical issues and the potential application of their findings to disease.

To enhance interaction among the CIRM trainees and to keep them up to date in this field, the Caltech program will include new stem cell seminar and journal club programs, as well as an annual symposium.

Stem Cell Training Grant
Childrens Hospital Los Angeles Program Director: Donald Kohn, M.D.

This Level II Training Grant will support seven PhD Post-Doctoral and three MD Clinical Fellows at Childrens Hospital Los Angeles (CHLA) for training in stem cell biology, and the clinical and ethical implications of stem cell research. CHLA's research has been consistently ranked in the top six of the nation's pediatric stand alone institutions by the NIH. Over the past 20 years, CHLA has built an internationally renowned research program in stem cell biology and its clinical applications. The program was founded on the fields of human hematopoietic stem cell biology, transplantation and gene therapy. In the past decade, the program has been expanded to include somatic stem cells from lung, pancreas, liver, gut, bladder and mesenchyme. In the past three years, investigators at CHLA have developed expertise in human embryonic stem cells (hESC) and established a hESC training and tissue culture core for CHLA investigators. A unique focus of the Training Program at CHLA will be the application to pediatric disorders such as diabetes, monogenic inherited disorders (cystic fibrosis, muscular dystrophy, sickle cell disease, etc), and congenital birth defects. It is our central hypothesis that childhood disorders will be especially responsive to therapies produced by the use of stem cells; advances in the use of stem cells to treat childhood illnesses will then lead the way to treatments for the many disorders that occur later in life. The training program includes: a didactic course on "Stem Cell Biology, Research Methods and Stem Cell Therapies", a course titled "The Ethics of Stem Cell Research and Therapies", participation in multiple existing training activities at CHLA, training in laboratory methods in hESC and joint participation in a didactic course taught by scientists from CHLA, USC Keck School of Medicine and the California Institute of Technology. A Steering Committee with stem cell researchers, clinical physician/scientists and medical educators will oversee selection and supervision of trainees, the mentoring process, and other activities of the training program.The biomedical environment and strength of stem cell research at CHLA combine to provide a rich milieu for training the next generation of physicians and scientists who will use stem cells as the basis for research and therapy.

Gladstone CIRM Scholar Program
The J. David Gladstone Institutes Program Director: Robert Mahley, M.D., Ph.D

The Gladstone CIRM Scholars Training Program will train CIRM scholars in the postdoctoral and clinical tracks. The J. David Gladstone Institutes conducts basic research on three of the most important medical problems of modern times: cardiovascular disease, AIDS, and neurodegenerative disorders. Each of these research areas addresses promising targets for regenerative medicine. Gladstone recently consolidated its research activities in a new 200,000 sq. ft. facility, including laboratory space constructed without federal funding. Its location—adjacent to the Mission Bay campus of the University of California, San Francisco (UCSF)—provides an ideal environment for Gladstone CIRM scholars to collaborate with leading researchers at Gladstone and in neighboring UCSF laboratories. The Gladstone program features two key mentors outside of Mission Bay: Dr. Joseph McCune, a Gladstone investigator at San Francisco General Hospital, and Dr. Pieter de Jong of Childrens Hospital of Oakland Research Institute (CHORI). Gladstone is an independent research institute affiliated with UCSF, and we are combining some of our educational activities with the robust training programs in stem cell biology at CHORI, UCSF, and UC Davis, thus facilitating synergy and eliminating duplication (as allowed in the RFA). Gladstone offers a unique training for CIRM scholars, providing a commitment to educating the next generation of biomedical scientists, highly interactive research groups, and substantial individual feedback from experienced mentors. More than 300 fellows have trained at Gladstone. In a national survey by The Scientist, Gladstone was rated as the 12th best place for postdoctoral scientists to work in the U.S. in 2005 (123 universities/institutions were ranked). The Gladstone CIRM Scholars Training Program will allow us to

enhance our research program to help meet the goals of regenerative medicine, while at the same time enhancing the training of young scientists.

Training in the Biology of Human Embryonic Stem Cells and Emerging Technologies
The Salk Institute for Biological Studies Program Director: Juan Izpisua Belmonte, Ph.D.

This is a Type III CIRM Training Proposal for 6 postdoctoral fellows to be educated at the Salk Institute. This Program is designed to develop and enhance research opportunities for postdoctoral fellows training for careers in the field of human stem cell biology. Our goals are to develop a curriculum of study and research experiences necessary to provide high quality research training and to ensure a continuing supply of well-trained scientists prepared to conduct cutting-edge health-related research in human embryonic stem cell biology. The rationale for this Training Program is that a deep understanding of the biology of human embryonic stem cells will be essential for utilizing them successfully to develop new therapies for human diseases. For this purpose we suggest a full range of multi-disciplinary training activities that range from the study of basic principles of stem cell biology, encompassing genetic, biochemical, and cellular approaches, to theoretical and practical aspects of stem cell related emerging technologies, to ethical, legal, and social issues involved with stem cell research, to colloquiums, lectures and seminars, with the ultimate goal of providing a well rounded training experience in the field of human embryonic stem cell biology. Training in research has been a key activity of the Salk Institute since its inception, reflected in the fact that many of the more than 2,000 scientists trained at the Institute have gone on to positions of leadership in other prominent research centers worldwide, including five Nobel prizes. This proposal aims to continue this record of achievement by capitalizing on the multi-disciplinary range of conceptual and methodological expertise present at the Institute in the biomedical field, that will be enhanced by the close research and training collaborative activities with the nearby institutions, UCSD, the Scripps Research Institute and the Burnham Institute.

Training Stem Cell Researchers at the Chemistry-Biology Interface
Scripps Research Institute Program Director: Peter Schultz, Ph.D.

We will exploit the unique strengths of The Scripps Research Institute (TSRI) in Chemistry and Biology to provide an interdisciplinary stem cell training program that incorporates teaching and research in chemistry, functional genomics, and molecular genetics. The goal of this proposal is to train scientists for future careers in basic or applied research in the field of stem cell biology. In particular, the aim of this program is to train coworkers who can work at the interface of chemistry and biology in order to more effectively apply chemical tools and approaches to basic research and the development of new therapeutic approaches in regenerative medicine. This requires a training program that brings together graduate students and postdoctoral fellows from the biology and chemistry disciplines in order to (1) educate them in the basic biology, methods, and applications in embryonic and adult stem cell biology; (2) cross train them in the principles and approaches that chemists and biologists apply to biological problems; (3) foster research collaborations between chemists and biologists in the stem cell field; and (4) stimulate an awareness of the problems and ethical issues associated with basic and applied stem cell research. This training program, although relatively small in size, will benefit from close nteractions with the broader San Diego stem cell community (UCSD, Salk, Burnham) including collaborative research projects and joint seminar programs, classes, and workshops. We are requesting a Type II program with support for 10 trainees to be educated at TSRI.

Stanford CIRM Training Program

Stanford University Program Director: Michael Longaker, M.D., M.B.A.

Stanford University is applying for three years of funding to establish an integrated CIRM Scholar training program in the fundamental biology of embryonic and adult stem cells. We aim to produce leaders who are positioned to understand basic stem cell mechanisms and to provide the fundamental and practical basis for the development of novel molecular and cellular therapies. We plan a 3 level Type I comprehensive training program with predoctoral (n=6), post-doctoral (n=5), and clinical fellows (n=5), for 16 concurrent CIRM Scholar positions. Stanford offers outstanding opportunities for training both MD and PhD predoctoral students, Ph.D. postdoctoral fellows, and clinical fellows in stem cell biology, regenerative medicine, and human disease. With the School of Medicine, Stanford Hospitals, and the University on one campus, Stanford brings a powerful combination of assets to this mission. In addition, Stanford faculty have extensive experience in basic research, clinical translation, and training in stem cell biology and medicine, including leading discoveries in tissue and organ stem cells, embryonic stem cells and cancer stem cells. We propose an integrated program of training that will bring together leading mentors and top trainees in basic science, engineering, and clinical medicine related to stem cells. The required coursework will include a new course on Social, Legal and Ethical Issues and Implications in Stem Cell Research, a new weekly colloquium alternating presentations of data by students and postdocs with seminars by Stanford faculty and prominent outside invited speakers, and a course on basic science and clinical correlates in human disease. Special features of the training program will include cross-disciplinary co-mentors and, for PhD scholars, a two week clinical immersion focused on a relevant clinical problem, application, or disease that may be a possible future target for stem cell therapy. The courses, seminars, annual retreats, journal clubs, and data clubs planned as part of this training program in stem cell biology and regenerative medicine will serve as a catalytic resource for a much greater number of students, fellows, and basic science and clinical faculty across the disciplines, from Biochemistry to Law, Developmental Biology to Engineering, and Business to Medicine.

Human Stem Cell Training at UC Berkeley and Childrens Hospital at Oakland

University of California, Berkeley Program Director: Randy Schekman, Ph.D.

UC Berkeley and the Childrens Hospital Oakland Research Institute (CHORI) propose training opportunities in stem cell biology and technology, their application to the treatment of disease, and the legal and ethical issues surrounding the study and use of stem cells. Capitalizing on the new opportunities in human stem cell science, faculty and staff have organized a stem cell center to consolidate our research activities in gene expression, tissue engineering, and the analysis of cord blood stem cells. We have designed a program to support the education and training of fellows in the full spectrum of issues relating to stem cell technology. Scientific coursework will cover renewal and differentiation of embryonic and somatically derived stem cells, the engineering of stem cells and tissues, and the characterization and therapeutic application of stem cells derived from human cord blood. This program will be complemented by teaching from faculty in the humanities, social sciences, and law, who will provide a comprehensive overview of sociological, bioethical and legal issues surrounding stem cell research. The training program will include 16 fellowships; 2 clinical fellows in pediatric hematology/oncology, 6 postdoctoral and 6 predoctoral fellows in molecular biology or bioengineering, 1 predoctoral fellow in humanities, and 1 fellowship in law, to be shared between two students. Science fellows' research will be in one of four major areas; growth, renewal and differentiation of hematopoietic and neural stem cells; engineering synthetic environments that control stem cell self-renewal and fate; gene networks in stem cell differentiation; and stem cells and cancer. Humanities fellows' research will promote the representation of women and minorities in stem cell research or combat health disparities in the development, testing and affordability of stem cell therapeutics. Law fellows will focus their studies on

the legal rules, issues and institutions most likely to impact the pursuit and application of stem cell research. This program will develop scholars with varying areas of expertise, but a shared appreciation of the scientific, ethical and legal complexities surrounding this emerging technology.

UC Davis Stem Cell Training Program
University of California, Davis Program Director: Fredrick Meyers, M.D.

The UC Davis Type I Stem Cell Training Program establishes a collaborative training experience dedicated to meeting the goals of the California Institute of Regenerative Medicine (CIRM). The overarching objective is to provide CIRM Scholars with state-of-the-art multidisciplinary team training to position them to become technically skilled, critically thinking, and collaborative scientists with successful independent research careers in stem cell biology and medicine. The training program will enroll pre-doctoral and post-doctoral students and clinical fellows (16 trainees/year). A talented pool of applicants will be selected from established graduate and clinical training programs. A formal application process overseen by an Internal Executive Committee is described, including standardized selection criteria. Faculty committed to the stem cell training program will be drawn from the UC Davis medicine, veterinary medicine, engineering, biological sciences, agriculture and environmental sciences, law, and management programs (13 lead mentors; 46 total mentors identified). CIRM Scholars will participate in (1) structured mentored research experiences; (2) core curriculum including courses in basic research skills; ethical, legal, and social implications of stem cell research; stem cell biology and medicine; and leadership training; (3) elective didactic and laboratory rotations; (4) special experiences (journal club, stem cell seminar series, symposia, annual retreat); and (5) further opportunities such as special laboratory rotations, field trips, interaction with the technology transfer office, and scholar presentations to K-12 classes and the lay public. The program leaders, Internal Executive Committee, and External Advisory Board will oversee program administration; assure implementation of training program objectives; facilitate recruitment, selection, retention and training; and evaluate scholars and mentors. Unique attributes of our training program include the California National Primate Research Center; biomedical engineering/in vivo imaging; collaboration with Shriner's Hospital, UC Merced, and Lawrence Livermore National Laboratory; robust combination of campus schools; and a strong commitment to diversity and outreach, all of which add significant value to the UC Davis research and training experience for the proposed CIRM Scholars.

Stem Cell Research Training Grant
University of California, Irvine Program Director: Peter Bryant, Ph.D.

We propose a Type I training program in Stem Cell Biology for eight predoctoral, four postdoctoral and four clinical CIRM Scholars, to be administered by the Stem Cell Research Center at the University of California, Irvine. Predoctoral Scholars will enter the program at the end of their first year in the Molecular Biology, Genetics and Biochemistry (MBGB) graduate program, the Interdepartmental Neurosciences Program (INP) or other relevant PhD program at UCI. CIRM pre- and post-doctoral Scholars will participate in research training in the labs of UCI stem cell mentors. The CIRM clinical Scholars will enter a new track in our existing residency and a subspecialty fellowship training program: after completing two years of clinical training in a specialty residency program and one year of clinical training in subspecialty fellowship program, the trainee will spend two years in basic or translational stem-cell research. All CIRM Scholars will participate, during their first year of support, in a year-long sequence of three new courses: Basic Biology of Stem Cells; Clinical Applications of Stem Cells; and Social, Legal and Ethical Implications of Stem-Cell Research. Trainees will also be provided with access to a specialized laboratory course entitled Human Embryonic Stem Cell Culture Training Course, a dedicated workshop on Large-Animal Models for Stem Cell Research, and seminars and symposia focused on stem cell biology and clinical application. We will provide a research training environment for Scholars in many

areas of stem cell biology including the Basic Biology of Stem Cells, Developing Tools for Stem Cell Research, Genetics of Cortical Stem Cells, CNS Injury and Stroke, Neurodegenerative Disease, Tissue Engineering, Diabetes, the Role of Stem Cells in Cancer, Stem-Cell Therapy for Ocular Disease, and Stem Cell-Based Screens to Identify Novel Drugs targeting cell proliferation, differentiation, and death. By formalizing, expanding and integrating stem cell training at UCI, we plan to contribute to the acceleration of stem cell research in the state, and to increase the number of scientists and clinicians participating in stem cell research and its application to treatments.

CIRM Comprehensive Training Program
University of California, Los Angeles Program Director: Owen Witte, M.D.

The aim of this UCLA Type I. Comprehensive Training Program is to train basic scientists, engineers, and physicians to become leaders in stem cell research and clinical programs in cademia and industry. A distinctive feature of the UCLA program is that Scholars will be trained from a multidisciplinary perspective, which is possible because faculty from the College of Letters and Science, and the Schools of Dentistry, Engineering, Law, Nursing, Medicine, and Public Affairs are located in close proximity on the same campus and have developed a tradition of multi-disciplinary teaching and research collaboration. The recently established UCLA Institute for Stem Cell Biology and Medicine (ISCBM) has been built on this foundation and has received strong campus-wide support as eVidenced by the allocation of 12 new faculty positions in stem cell biology and a major space commitment. The ISCBM will coordinate the training of 5 pre-doctoral, 5 postdoctoral, and 6 clinical Scholars, each of whom will be presented with numerous training options. Some may choose to work with UCLA faculty who are leaders in cell and molecular biology, while others will elect to receive training in gene medicine, cell-based therapies, and organ transplantation from clinician-scientists already applying these new approaches to patient care. As part of this training Scholars will develop an understanding of Good Manufacturing Practices (GMP) and compliance issues related to clinical trials. Scholars with interests in bioengineering and nanotechnology can work with faculty developing methods for manipUlating and Visualizing cells using microfluidic systems, bio-mechanical tools, and nano-scale microscopes or whole body imagers. The training program will also accommodate Scholars interested in social, legal, or policy aspects of stem cell research. In addition to their individualized research program, all Scholars will participate in numerous common activities, including a new course in Stem Cell Biology that includes lectures and discussion sessions on adult and embryonic stem cells (ESC), organogenesis, tissue repair, nuclear reprogramming, gene therapy, the conduct of clinical trials, and social, legal, and ethical aspects of stem cells. Scholars will also have the opportunity to join one or more journal clubs, attend research seminars featuring leading stem cell biologists, and present talks describing their own research. Regardless of their particular interest, by the end of their training, Clinical Scholars, many of whom are simultaneously working for a graduate degree and board certification through the UCLA STAR Program, will obtain an appreciation of basic research and basic scientists and engineers will acquire knowledge of human disease and an understanding of how research advances are translated to the clinic. Whether they ultimately work individually or as part of a multidisciplinary team, the UCLA program will train highly skilled scientists and clinicians who will help to make the practice of regenerative medicine a reality.

Interdisciplinary Stem Cell Training Program at UCSD
University of California, San Diego Program Director: Lawrence Goldstein, Ph.D.

This proposal describes a Type I stem cell training program at UCSD including the UCSD School of Medicine, the UCSD Division of Biology, the UCSD Skaggs School of Pharmaceutical Sciences, and The UCSD Jacobs School of Engineering. This program is designed to provide interdisciplinary training in stem cell biology and medicine by taking advantage of the unique interdisciplinary and inter-institutional environment at UCSD and in La Jolla. A major goal is to train a cadre of young basic and clinical

scientists and engineers in the use of quantitative and engineering approaches from the physical sciences such as chemistry, computation, and materials science to make novel discoveries in basic and clinical biomedicine. Basic and clinical science and engineering trainees who complete our program will be ideally suited for future careers as academic or industrial scientists investigating stem cell biology and medicine, or using stem cell based methods to develop new therapeutic approaches to human diseases. Our approach will be to build on each trainees' specialized foundation of basic or clinical knowledge and provide: 1) Rigorous education in the principles and applications of embryonic and adult stem cell biology from humans and model organisms; 2) Research training in physical, computational, and engineering methods that can be used to harness stem cells to attack problems of basic and clinical science and their uses to develop new understanding and new therapies; and 3) Education in the problems and outlooks associated with the ethical, legal, social, and economic issues associated with stem cell biology. Our training program will also serve as a catalyst for the integration of our expanding stem cell biology research and training efforts at UCSD and on the La Jolla Mesa. Thus, we are requesting financial support for 16 trainees (6 graduate, 4 postdoctoral, 6 clinical fellow).

Training Program in Stem Cell Research at UCSF
University of California, San Francisco Program Director: Susan J. Fisher, Ph.D.

Stem cell research holds great promise for developing cell-based therapies for common diseases. Scientists at UCSF have contributed new insights that have enhanced our understanding of stem cell biology, including identification of neural stem cells, derivation and characterization of greatly-improved human embryonic stem cell lines, and application of innovative islet transplantation methods to treat diabetes. These discoveries highlight the need for scientists who can bridge basic and clinical sciences to fully realize the clinical potential of stem cells. The goal of the UCSF Training Program in Stem Cell Research is to train CIRM scholars in basic research who are cognizant of clinical needs, as well as scholars in clinical disciplines who are grounded in the basic science of stem cell research. We will achieve this long-term goal by: (1) capitalizing on our strong, multidisciplinary faculty engaged in stem cell-related research to provide training in neural stem cells, cardiac repair and regeneration, angiogenesis, diabetes, developmental biology, hematopoiesis and cancer stem cells, mesenchymal biology, bioengineering, and human embryonic stem cells; (2) exploiting our world-class graduate programs to recruit talented pre-doctoral students to stem cell research; (3) attracting top PhD and MD/PhD graduates to postdoctoral careers in stem cell research at UCSF; (4) providing a mechanism for outstanding clinical fellows to obtain rigorous training in basic stem cell research. This program will include three major training components: (1) didactic coursework in developmental and stem cell biology, embryology, human disease and transplantation; (2) training in the rigorous ethics required to address difficult questions surrounding stem cell research and regenerative medicine; and (3) a mentored research program under the guidance of leading basic science and/or clinical stem cell investigators as preparation for a career aimed toward treating human diseases. Training will occur in an environment enriched by opportunities to present research, attend journal clubs and retreats and learn from outstanding scientists through formal seminars. We will also extend our courses and training to those who attend neighboring institutions in order to drive stem cell research forward and train the next generation of stem cell scientists, clinicians and technicians.

UCSB Stem Cell Biology Training Program
University of California, Santa Barbara Program Director: Dennis Clegg, Ph.D.

Human embryonic stem cells (hES cells) are immortal pluripotent cells with the potential to differentiate into derivatives of all three germ layers. They offer enormous potential in the emerging field of regenerative medicine and for illuminating basic developmental biology in vitro, although regulatory mechanisms that control their differentiation are not understood. Despite this potential, due to the fact that

they originate from preimplantation embryos, they have generated great ethical controversy. To help young scientists sort out fact from fiction, and to offer them training as well as ethical guidance in hES research, we propose a level III training program for pre and post-doctoral scientists. The goal of the training program is twofold: 1) to teach young scientists to think critically and independently about hES cell research and 2) to apply this knowledge to the practical applications of hES cells in regenerative medicine. The focus of research will be an interdisciplinary approach to understanding how hES differentiation can be regulated to produce ocular cells, which might be useful in the treatment of eye disease. The research program will take advantage of unique strengths present at UCSB in the Department of Molecular, Cellular and Developmental Biology, the Neuroscience Research Institute, the Center for the Study of Macular Degeneration, and the College of Engineering. Our researchers and collaborators have published the first paper on human retinal cells derived from hES cells and are now testing them in preclinical animal models of retinal disease. We also have assembled a world class team of ethicists to teach the young scientists how to judge and make the appropriate moral and ethical decisions they may encounter in this area of research. This training program should prepare the trainees for a rewarding career in stem cell research and provide them with a rare early opportunity to see this basic research translated into actual animal models of human disease.

Training Program in Systems Biology of Stem Cells
University of California, Santa Cruz Program Director: David Haussler, Ph.D.

We propose to develop a Type III Specialized Training Program, to include 6 trainees - 3 at the predoctoral level and 3 at the postdoctoral level. Drawing from UCSC's strengths in computational genomics and basic biological research, our "Training Program in Systems Biology of Stem Cells" will provide students with a solid understanding of the biology of stem cells, the skills to utilize stem cells in their own research, and the ability to devise computational approaches and to integrate results from computational analyses into their own work.

The program will underscore the value of stem cell research in developing therapies and cures for human disease. UCSC faculty have exceptional expertise in areas of research that are critical for advancing stem cell research, including genome sequence analysis, RNA biology, cell cycle reguiation, early development, neuroscience, and epigenetic mechanisms of gene regulation such as chromatin remodeling. We are particularly well-suited to apply engineering approaches to challenges in stem cell research - many of our faculty regularly work across the divisional boundaries between science and engineering - and to train CIRM Scholars from broad backgrounds at this interface. Furthermore, as one of the premier public institutions for humanities and social science research, we are proposing a stem cell ethics course that will be exceptional in the range and depth of issues covered. We are developing this program in parallel to programs being developed at UC Berkeley, UC San Francisco, and the Gladstone Institutes. We intend to share certain courses and other activities such as joint seminars and symposia. We advocate the formation of a Bay Area Stem Cell Alliance and have been in early discussions about such an organization with some of our neighboring institutions. We will also provide a number of on campus opportunities for enriching the training experiences of CIRM Scholars, including a seminar series, a journal club, an annual retreat, and a web page devoted to news, events and opportunities related to stem cell research.

CIRM Stem Cell Biology Training Grant
University of Southern California Program Director: Robert Maxon, Ph.D.

This is a proposal for a Type I Comprehensive Training Program in Stem Cell Biology to be based at the University of Southern California (USC). Our program will train post-doctoral and clinical fellows across 27 departments at USC. Pre-doctoral trainees will be recruited from 11 Ph.D. programs in the Schools of

Medicine and Gerontology and in the College of Letters, Arts and Sciences. We have assembled a team of world-class scientists to teach two new courses developed through this initiative: an interdisciplinary, USC-based course in the social, legal and ethical implications of stem cells, and a joint course among USC, its affiliate, Childrens Hospital Los Angeles, and the California Institute of Technology in stem cell biology. Each of these institutions is deeply committed to the involvement of students and faculty in this program beyond the scope of this funding. A strength of our program will be a high degree of interaction among students, 26 mentors and 36 instructors spanning all three institutions and facilitated by in-person, video, and web-based exchanges. We are requesting funding through this mechanism in order to recruit nationally 16 highly qualified individuals by the second year to be funded and designated as CIRM Scholars. In addition, these funds will support seminars and retreats that will build a larger community for stem cell research by involving and facilitating collaboration among students and faculty.

CHAPTER 2

APPROVED APPLICATIONS FOR HUMAN EMBRYONIC STEM CELL RESEARCH BY INDIVIDUAL INVESTIGATORS

In 2006, CIRM implemented two grant programs to support proposals for individual investigator-initiated research. The Leon J. Thal SEED Grants are intended to bring new ideas and new investigators into the field of human embryonic stem cell research. They offer an opportunity to conduct studies that may yield preliminary data or proof-of-principle results that could then be extended to full scale investigations. The Comprehensive Research Grants are intended to support mature, ongoing studies on human embryonic stem cells by scientists with a record of accomplishment in the stem cell field.

The objective of these programs, to attract both newly independent investigators and seasoned scientists to develop innovative research projects in human embryonic stem cell research, was met successfully. Of those selected for funding in the Leon J. Thal SEED program, 42% of the applicant scientists are new to the field of stem cell research and 37% are newly independent investigators. In the Comprehensive Research Grant program, 48% of successful applicants are established stem cell researchers planning to use human embryonic stem cells in their laboratories for the first time, and 24% are newly independent investigators who have experience working with human embryonic stem cells.

Both programs support the use of human embryonic stem cells in projects designed either to shed light on basic biological processes believed to be essential for future development of stem cell applications, or to address directly the treatment or cure of specific diseases and injuries. Many projects focus on novel techniques and approaches for producing fully functional, mature cells that could be used in cell replacement therapy, to create cell culture models of human diseases or to screen for new drugs. Of 103 applications for individual awards that were approved for funding in 2007, 57 projects relate to specific diseases and injuries and 46 projects focus on the biology of human embryonic stem cells. The research projects encompass a wide range of diseases for which human embryonic stem cells may provide a treatment or cure.

APPROVED APPLICATIONS RELATED TO SPECIFIC DISEASES AND INJURIES

CONDITIONS OF THE BLOOD AND THE IMMUNE SYSTEM

The main role of the immune system is to combat infection and disease, an essential function for survival. Immune function can be severely compromised by challenges such as treatments for cancer, infection by specific viruses such as those associated with AIDS and other diseases, and even by the aging process. Moreover, an individual's own immune cells can go awry and cause blood and autoimmune diseases (such as leukemia and rheumatoid arthritis). Hematopoietic stem cells residing in bone marrow are the source of all the specialized cells of the blood and immune system and can provide a life-saving treatment for some diseases. Nevertheless, hematopoietic stem cells are quite rare and donor cells must be matched to the patient to be tolerated; often matched donors cannot be found. Currently there is no stem cell line that works as a 'universal donor', accepted by the immune systems of all patients. Therefore, learning how to purify, grow, and manipulate human embryonic stem cells to behave and be tolerated like a recipient's own hematopoietic stem cells is an important and active area of research. In 2007, eight awards were approved for funding projects dedicated to the eventual prevention, treatment or cure of hematopoietic disorders, HIV-associated disease or rejection of transplants.

HEMATOPOIETIC DISEASES

LEON J. THAL SEED GRANT PROGRAM

Improving microenvironments to promote hematopoietic stem cell development from human embryonic stem cells
University of California, Los Angeles
P.I.: Hanna Mikkola, M.D., Ph.D.
$580,000 for 2 years

Generation of long-term cultures of human hematopoietic multipotent progenitors from embryonic stem cells
University of California, San Diego
P.I.: Cornelis Murre, Ph.D.
$540,000 for 2 years

Embryonic stem cell-derived thymic epithelial cells
Stanford University
P.I.: Kenneth I. Weinberg, M.D.
$660,000 for 2 years

Dollar amount shown is the amount requested rounded to the nearest $5,000, not the amount awarded.

COMPREHENSIVE RESEARCH GRANT PROGRAM

Embryonic stem cell-derived thymic epithelial cells
Stanford University
P.I.: Irving L. Weissman, M.D.
$2,640,000 for 4 years
Also referenced: Transplantation Tolerance

HUMAN IMMUNODEFICIENCY VIRUS (HIV) ASSOCIATED DISEASE

LEON J. THAL SEED GRANT PROGRAM

Genetic modification of the human genome to resist HIV-1 infection and/or disease progression
University of California, Los Angeles
P.I.: Irvin S.Y. Chen, Ph.D.
$645,000 for 2 years

COMPREHENSIVE RESEARCH PROGRAM

Human Embryonic Stem Cell Therapeutic Strategies to Target HIV Disease
University of California, Los Angeles
P.I.: Jerome A. Zack, Ph.D.
$2,520,000 for 4 years

TRANSPLANTATION TOLERANCE

LEON J. THAL SEED GRANT PROGRAM

Down-Regulation of Alloreactive Immune Responses to hES Cell-Derived Graft Tissues
University of California, Los Angeles
P.I.: Noriyuki Kasahara, M.D., Ph.D.
$470,000 for 2 years

COMPREHENSIVE RESEARCH PROGRAM

Embryonic stem cell-derived thymic epithelial cells
Stanford University
P.I.: Irving L. Weissman, M.D.
$2,640,000 for 4 years
Also referenced in: Hematopoietic Diseases

Dollar amount shown is the amount requested rounded to the nearest $5,000, not the amount awarded.

ABSTRACTS

[Provided by applicant]

HEMATOPOIETIC DISEASES

Improving microenvironments to promote hematopoietic stem cell development from human embryonic stem cells

University of California, Los Angeles
Principal Investigator: Hanna Mikkola, M.D., Ph.D. Leon J. Thal SEED Grant Program

Hematopoietic stem cells (HSC) have been used successfully to cure various life-threatening blood diseases. Yet, matching HSCs are not available for every patient. Human embryonic stem cells (hESC) may provide an unlimited source of HSCs for therapeutic use. However, hESC derived hematopoietic cells do not develop properly in those culture conditions that are currently used, and thereby their function is impaired. Hematopoietic cells that are derived from human ES cells lack the ability to self-renew, which is a prerequisite for the ability to generate blood cells for the individual's lifetime. HSCs can only develop and function normally if they receive correct signal from their microenvironment, the stem cell niche. The goal of our proposal is take advantage of our knowledge of development of hematopoietic stem cells during embryogenesis, and mimic the environments where HSCs normally develop to provide the cues for proper HSC development in culture.

We will attempt to mimic physiological HSC niches by deriving stroma lines from human placentas, which we have shown to be an important site for HSC development. We will further modify these lines by adding regulatory molecules that are known to aid HSC self-renewal, or inhibit molecules that might promote premature differentiation. Alternatively, we will use placental villi as a niche where ES cell derived hematopoietic cells could develop during culture. Subsequently, hESC derived cells are tested in animal models where human hematopoietic tissues have been implanted to provide an optimal environment for human HSCs to function.

These studies are expected to shed light on the mechanisms that enable human HSCs to establish and maintain self-renewal ability and multipotency, and improve the differentiation of hESCs towards functional HSCs, which could be used to treat leukemias, other cancers, and inherited disease of the blood and immune system. To ensure hESC lines derived in different conditions respond in a similar way to these developmental cues, non-federally approved lines have to be used in this study, and thus governmental funding is not attainable for this project (Hanna Mikkola, University of California, Los Angeles).

Generation of long-term cultures of human hematopoietic multipotent progenitors from embryonic stem cells

University of California, San Diego
Principal Investigator: Cornelis Murre, Ph.D. Leon J. Thal SEED Grant Program

For many therapeutic reasons it is important to have available large numbers of blood cells. However, it is difficult to generate large numbers of specialized blood cells that have the ability to neutralize autoimmunity and response to tumor cell growth. In this study we would develop a technique that would allow the production of large numbers of different types of blood cells from human embryonic stem cells. For example, a subset of white blood cells, called dendrititc cells, is currently manipulated in the laboratory in a manner that allows them to attack cancer cells. The same cells also are altered in the laboratory to counter-act the development of autoimmune diseases. A problem with these experiments is that it is difficult to isolate large numbers of these cells, since they are relatively rare. With the technology

that is described in this grant application we would be able to generate large numbers of such cells in the laboratory using as a starting point, human embryonic stem cells.

Embryonic stem cell-derived thymic epithelial cells
Stanford University
Principal Investigator: Kenneth I. Weinberg, M.D. Leon J. Thal SEED Grant Program

The function of the immune system throughout life is essential for protection from infections and cancer. T lymphocytes are white blood cells that choreograph the multiple responses that the body uses to control infection. T lymphocytes are produced in the thymus, a specialized organ located in the chest in front of the heart. The production of new T lymphocytes ('thymopoiesis') is abnormal in some children with genetic defects in the development of the thymus (DiGeorge syndrome [DGS]), but even in healthy people, thymic function declines with age. Thymic insufficiency, the decreased ability of the thymus to make new T lymphocytes, is a serious health problem. For example, if the T lymphocytes that have been previously made were to be destroyed by HIV infection, chemotherapy or radiation therapy, or hematopoietic stem cell transplantation, the restoration of immune function requires the production of new T lymphocytes to replace those that were lost. For this reason, adults with such conditions have poorer recovery of immune function than children and the elderly have increasing risk of severe infection with age. For example, 10-40% of the elderly do not respond to annual influenza vaccination and as many as 50-100,000 may die of influenza annually.

Thymic insufficiency is due to injury or death of cells called thymic epithelial cells (TEC). TEC resemble skin cells but produce a number of proteins such as interleukin-7 (IL-7) needed by developing T lymphocytes in the thymus ('thymocytes'). Like skin cells, TEC become more fragile and easily injured with age. Also like skin cells, TEC are destroyed by chemotherapy and radiation therapy. Clinical efforts to restore thymopoiesis in patients with HIV infection by transplantation of thymic tissue from unrelated donors have not been successful because of rejection of the transplanted tissue. Experimental efforts to correct the problem of decreased thymopoiesis have included attempts to replace TEC functions by injections of IL-7 or other cells that make IL-7; or to regenerate TEC by the injection of keratinocyte growth factor (KGF), a protein that stimulates the growth of TEC.

Human embryonic stem cells (hESC) are a potential source of replacement TEC that could be used to regenerate the immune system in people whose pool of T lymphocytes has been decreased, e.g., the elderly, or those with HIV or cancer. In order to implement such a strategy, research on how to control the development of TEC from hESC are necessary. The proposed studies will test how certain growth factors and genes such as those defective in DGS control the development of TEC from hESC. In addition, the studies will develop model systems in mice for testing the ability of TEC to be transplanted, a necessary scientific tool for the assessment of future therapies that will use TEC progenitors to restore immune function.

Embryonic stem cell-derived thymic epithelial cells
Stanford University
Principal Investigator: Irving L. Weissman, M.D. Comprehensive Research Grant Program
Also referenced in: Transplantation Tolerance

The capacity of human embryonic stem cells (hESCs) to perpetuate themselves indefinitely in culture and to differentiate to all cell types of the body has lead to numerous studies that aim to isolate therapeutically relevant cells for the benefit of patients, and also to study how genetic diseases develop. However, hESCs can cause tumors called teratomas when placed in the body and therefore, we need to separate potentially beneficial cells from hazardous hESCs. Thus, potential therapeutics cannot advance until the

development of methodologies that eliminate undifferentiated cells and enrich tissue stem cells. In our proposal we hope to define the cell surface markers that are differentially expressed by committed hESC-derived stem cells and others that are expressed by teratogenic hESCs. To do this we will carry out a large screen of cell subsets that form during differentiation using a collection of unique reagents called monoclonal antibodies, many already obtained or made by us, to define the cell-surface markers that are expressed by teratogenic cells and others that detect valuable tissue stem cells. This collection, after filing for IP protection, would be available for CIRM investigators in California. We were the first to isolate mouse and human adult blood-forming stem cells, human brain stem cells, and mouse muscle stem cells, all by antibody mediated cell-sorting approaches. Antibody mediated identification of cell subsets that arise during early hESC differentiation will allow separation and characterization of defined subpopulations; we would isolate cells that are committed to the earliest lineage known to form multiple cell types in the body including bone, blood, heart and muscle. These cells would be induced to differentiate further to the blood forming and heart muscle forming lineages. Enriched, and eventually purified hESC-derived blood-forming stem cells and heart muscle stem cells will be tested for their potential capacity to engraft and improve function in animal models. Blood stem cells will be transplanted into immunodeficient mice to test their capacity to give rise to all blood cell types; and heart muscle stem cells will be transferred to mouse hearts that had an artificial coronary artery blockage, a model for heart attack damage. Finally, we will test the capacity of blood stem cell transplantation to induce transplantation tolerance towards heart muscle stem cells from the same donor cell line. Transplantation tolerance in this case means that the heart cells would be accepted as 'self' by the mouse that had it's unrelated donor immune system replaced wholly or in part by blood forming stem cells from the same hESC line that gave rise to the transplantable heart stem cells, and therefore would not be rejected by it's own immune system. This procedure would allow transplantation of beneficial tissues such as heart, insulin-producing cells, etc., without the use of immunosuppressive drugs.

HUMAN IMMUNODEFICIENCY VIRUS (HIV) ASSOCIATED DISEASE

Genetic modification of the human genome to resist HIV-1 infection and/or disease progression

University of California, Los Angeles
Principal Investigator: Irvin S.Y. Chen, Ph.D. Leon J. Thal SEED Grant Program

The proposed studies describe the genetic approaches utilizing human embryonic stem cells to suppress and/or eliminate the expression of the human protein CCR5. CCR5 is found on the surface of white blood cells. HIV-1 attaches to CCR5 and uses CCR5 to enter into its target cells. Our approach is to utilize established as well as new non-established approaches to prevent CCR5 from appearing on the surface of the cells. If CCR5 is not present, HIV-1 cannot infect the cells. Interestingly, this concept has already been proven in nature. Approximately 1% of the Caucasian population is genetically deficient for CCR5 and these individuals are resistant to HIV-1 transmission. Their white blood cells, when placed in culture, also resist HIV-1 infection in the laboratory. As such, we believe that our approach can be used to protect high risk individuals from HIV-1 infection as well as impede or stop progression of disease in those individuals already infected.

Human Embryonic Stem Cell Therapeutic Strategies to Target HIV Disease

University of California, Los Angeles
Principal Investigator: Jerome A. Zack, Ph.D. Comprehensive Research Grant Program

AIDS is a disease that currently has no cure. It arises when the human immunodeficiency virus (HIV) infects certain types of blood cells. These cells would normally be used to fight infection, but instead are

destroyed by the virus, leading to immunodeficiency. We have recently been able to induce the development of human embryonic stem cells (hESC) into the types of cells that HIV can infect. In addition, we were able to show that a marker gene could be introduced into the hESC, and this gene continued to produce its protein throughout development of the cell into the more mature blood cell types. This sets the stage for testing the possibility of using gene-modified hESC to treat HIV or other immune system diseases. We have 3 different types of anti-HIV genetic approaches that we will test in laboratory models. These will be placed into hESC, and the cells allowed to develop into blood cells. We will then test whether our 'therapeutic' genes can inhibit HIV infection in culture. We will also develop novel mouse models that allow development of hESC into blood cells in the body (in vivo). We will test the efficacy of certain of these genetic approaches in these systems, as they should more closely represent the situation in people. These studies will provide proof-of-principle that cells in the immune system can be modified by manipulation of hESC, and may help to develop future therapeutic approaches to combat HIV disease. In addition, these studies will be relevant to other immune system disorders such as autoimmune diseases.

It was estimated that by January 31 2005, approximately 151,000 Californians were HIV infected. Furthermore, according to the California HIV Surveillance Report, 1752 new cases of HIV infection (1700 adult and 52 pediatric cases), and 5 deaths were reported between April 1 and September 31, 2006. Current treatment strategies prolong life, but do not cure infection, and are themselves quite toxic. Consequently HIV disease, and improved therapeutic approaches for this disease, are issues of great importance to the people of California.

TRANSPLANTATION TOLERANCE

Down-Regulation of Alloreactive Immune Responses to hES Cell-Derived Graft Tissues

University of California, Los Angeles
Principal Investigator: Noriyuki Kasahara, M.D., Ph.D. Leon J. Thal SEED Grant Program

Human Leukocyte Antigens (HLA) are proteins that are expressed on the surface of almost all cells in the body. Because HLA sequences are highly variable and each person generally has a different set of HLA gene sequences, these cell surface markers serve as the identifiers of 'self' vs. 'non-self'. If immune cells in the body encounter foreign cells transplanted from a different individual, in most cases these foreign cells are recognized due to their display of a different 'non-self' HLA on their cell surfaces, and attacked by the immune system. However, because it is difficult to obtain donors with precise matches, many patients succumb to their disease while on a waiting list for matched bone marrow or organs. Even one mismatch in HLA can result in immune responses against the transplant graft, making it necessary to administer immunosuppressive drugs for the lifetime of the patient.

Initially it was thought that human embryonic stem cell (hESC)-derived cells and tissues might not be attacked by the immune system because these cells do not have much HLA on their surfaces in their primitive state. However, it is now known that once hESC start to develop into mature adult-type cells, they also start to increase their display of HLA, marking them as foreign 'non-self' transplants. Thus, for hESC-derived cell and tissue transplants face the same problem of immune rejection as adult organ transplants.

Gene therapy is a promising new treatment approach that involves the delivery of genetic material such as DNA or RNA directly into cells, thus altering their genetic configuration and 're-programming' them to change the pattern of cellular protein expression. Long-term genetic re-programming can be efficiently achieved with the use of certain types of virus, chiefly retroviruses, which insert themselves directly into

the chromosomes of the infected cell, becoming a permanent part of that cell's genome. Lentiviruses are a type of retrovirus which includes pathogens such as HIV, but as gene delivery vehicles ('vectors'), they have been completely disabled by removal of the viral genes, and replacing them with the therapeutic sequences we want them to deliver, thus turning viral foes into friends. We propose to use this approach to deliver a newly discovered class of 'small interfering RNA' (siRNA) that can be designed to target and down-regulate specific sequences in the cell, thereby silencing expression of specific genes such as HLA, without affecting other cellular proteins. Since the genetically re-programmed hESC-derived transplants will no longer display their own HLA due to siRNA-mediated silencing, this gene therapy approach may make it possible to create 'universal' donor cells by erasing the HLA identifiers completely, or at least may expand the usefulness of existing hESC-derived donor cells and tissues by nullifying certain subsets of HLA sequences and thus making it easier to find matches with the remaining HLA sequences.

Embryonic stem cell-derived thymic epithelial cells

Stanford University

Principal Investigator: Irving L. Weissman, M.D. Comprehensive Research Grant Program

Also referenced in: Hematopoietic Diseases

The capacity of human embryonic stem cells (hESCs) to perpetuate themselves indefinitely in culture and to differentiate to all cell types of the body has lead to numerous studies that aim to isolate therapeutically relevant cells for the benefit of patients, and also to study how genetic diseases develop. However, hESCs can cause tumors called teratomas when placed in the body and therefore, we need to separate potentially beneficial cells from hazardous hESCs. Thus, potential therapeutics cannot advance until the development of methodologies that eliminate undifferentiated cells and enrich tissue stem cells. In our proposal we hope to define the cell surface markers that are differentially expressed by committed hESC-derived stem cells and others that are expressed by teratogenic hESCs. To do this we will carry out a large screen of cell subsets that form during differentiation using a collection of unique reagents called monoclonal antibodies, many already obtained or made by us, to define the cell-surface markers that are expressed by teratogenic cells and others that detect valuable tissue stem cells. This collection, after filing for IP protection, would be available for CIRM investigators in California. We were the first to isolate mouse and human adult blood-forming stem cells, human brain stem cells, and mouse muscle stem cells, all by antibody mediated cell-sorting approaches. Antibody mediated identification of cell subsets that arise during early hESC differentiation will allow separation and characterization of defined subpopulations; we would isolate cells that are committed to the earliest lineage known to form multiple cell types in the body including bone, blood, heart and muscle. These cells would be induced to differentiate further to the blood forming and heart muscle forming lineages. Enriched, and eventually purified hESC-derived blood-forming stem cells and heart muscle stem cells will be tested for their potential capacity to engraft and improve function in animal models. Blood stem cells will be transplanted into immunodeficient mice to test their capacity to give rise to all blood cell types; and heart muscle stem cells will be transferred to mouse hearts that had an artificial coronary artery blockage, a model for heart attack damage. Finally, we will test the capacity of blood stem cell transplantation to induce transplantation tolerance towards heart muscle stem cells from the same donor cell line. Transplantation tolerance in this case means that the heart cells would be accepted as 'self' by the mouse that had it's unrelated donor immune system replaced wholly or in part by blood forming stem cells from the same hESC line that gave rise to the transplantable heart stem cells, and therefore would not be rejected by it's own immune system. This procedure would allow transplantation of beneficial tissues such as heart, insulin-producing cells, etc., without the use of immunosuppressive drugs.

CANCER

Stem cells are highly relevant to cancer research for identifying key molecules and cellular mechanisms of transformation, as a tool for drug discovery, and as potential treatments. Recent scientific reports indicate that some cells within tumors can be considered 'cancer stem cells', and may be responsible for the ability of cancer cells to continually divide and grow tumors. Five projects that are relevant to understanding cancer as well as diagnosis and treatment are approved for funding in 2007.

LEON J. THAL SEED GRANT PROGRAM

Genetic Enhancement of the Immune Response to Melanoma via hESC-derived T cells
University of California, Los Angeles
P.I.: Zoran Galic, Ph.D.
$645,000 for 2 years

Derivation and Characterization of Cancer Stem Cells from Human ES Cells
University of California, San Diego
P.I.: Catriona Jamieson, M.D., Ph.D.
$645,000 for 2 years

hESC as tools to investigate the neural crest origin of Ewing's sarcoma
Childrens Hospital of Los Angeles
P.I.: Elizabeth R. Lawlor, M.D., Ph.D.
$680,000 for 2 years

Using human embryonic stem cells to treat radiation-induced stem cell loss: Benefits vs cancer risk
University of California, Irvine
P.I.: Charles L. Limoli, Ph.D.
$630,000 for 2 years

COMPREHENSIVE RESEARCH GRANT PROGRAM

Immunology of neural stem cell fate and function
Stanford University
P.I.: Theo D. Palmer, Ph.D.
$2,505,000 for 4 years

Dollar amount shown is the amount requested rounded to the nearest $5,000, not the amount awarded.

ABSTRACTS

[Provided by applicant]

Genetic Enhancement of the Immune Response to Melanoma via hESC-derived T cells
University of California, Los Angeles
Principal Investigator: Zoran Galic, Ph.D. Leon J. Thal SEED Grant Program

The overall goal of the proposed studies is to utilize human gene therapy approach using human embryonic stem cells to direct our body's defenses to specifically attack melanoma tumor cells. Current technologies try to accomplish this by genetically manipulating certain circulating T lymphocytes, such that they will target tumor cells. T lymphocytes are the major cell type of our body's immune system. However it is likely that this type of approach will not result in the presence of stable, lifelong genetically modified T cells. In contrast, a potentially more long-lasting approach would be to genetically modify human embryonic stem cells with the same therapeutic gene. Stem cells have the ability to form any type of blood cell, including T cells. Importantly, stem cells can persist for the life of the individual, and thus have the potential to produce genetically modified T cells for many years. In addition, these new tumor specific cells should expand in the body in response to the presence of the tumor, thus a large supply of tumor-fighting cells should be available as long as needed. This project proposes to develop novel means to introduce the anti-cancer gene into human embryonic stem cells. These stem cells will then be differentiated to generate tumor specific T cells utilizing animal model systems. We will then use several laboratory and mouse models to determine if the T cells derived from these genetically modified stem cells have anti-tumor activity. If successful, we will have provided proof-of principle that long-lived stem cells have the potential be utilized as a means of producing anti-cancer T cells. In the long run, these results could provide important information for design of future clinical trials designed to produce life-long improved anti-cancer immune responses.

Derivation and Characterization of Cancer Stem Cells from Human ES Cells
University of California, San Diego
Principal Investigator: Catriona Jamieson, M.D., Ph.D. Leon J. Thal SEED Grant Program

Cancer is the leading cause of death for people younger than 85 (1). High cancer mortality rates underscore the need for more sensitive diagnostic techniques as well as therapies that selectively target cells responsible for cancer propagation (1) Compelling studies suggest that human cancer stem cells (CSC) arise from aberrantly self-renewing tissue specific stem or progenitor cells and are responsible for cancer propagation and therapeutic resistance (2-9). Although the majority of current cancer therapies eradicate rapidly dividing cells within the tumor, the rare CSC population may be quiescent and then reactivate resulting in disease progression and relapse (2-9). We recently demonstrated that CSC are involved in progression of chronic phase chronic myelogenous leukemia (CML), a disease that has been the subject of many landmark discoveries in cancer research(19-30), to a more aggressive and therapeutically recalcitrant myeloid blast crisis (BC) phase. These CSC share the same cell surface markers as granulocyte-macrophage progenitors (GMP) but have aberrantly gained the capacity to self-renew as a result of activation of the Wnt/+-catenin stem cell self-renewal pathway (4). Because human embryonic stem cells (hESC) have robust self-renewal capacity and can provide a potentially limitless source of tissue specific stem and progenitor cells in vitro, they represent an ideal model system for generating and characterizing human CSC (10-18). Thus, hESC cell research harbors tremendous potential for developing life-saving therapy for patients with cancer by providing a platform to rapidly and rationally test new therapies that specifically target CSC (2-18).

To provide a robust model system for screening novel anti-CSC therapies, we propose to generate and characterize CSC from hESC (10-18). We will investigate the role of genes that are essential for initiation of CML such as BCR-ABL and additional mutations such as b-catenin implicated in CSC propagation (19-30). The efficacy of specific Wnt/b-catenin antagonists at inhibiting BCR-ABL+ human ES cell self-renewal, survival and proliferation alone and in combination with potent BCR-ABL antagonists will be assessed in sensitive in vitro and in vivo assays with the ultimate aim of developing highly active anti-CSC therapy that may halt cancer progression and obviate therapeutic resistance (4,31).

hESC as tools to investigate the neural crest origin of Ewing's sarcoma

Childrens Hospital of Los Angeles
Principal Investigator: Elizabeth R. Lawlor, M.D., Ph.D. Leon J. Thal SEED Grant Program

Human embryonic stem cells (hESC) hold great promise as sources of tissue for regenerative medicine and therapeutics. In addition, their utility as tools to study the origins and biology of human disease must not be underestimated. hESC give rise to tissue-specific adult stem cells and, ultimately, to all mature tissues in the body. As such, disruptions to normal stem cell function can have catastrophic consequences and result in life-threatening or debilitating disease. Understanding how such diseases arise will afford novel insights into how we can better prevent and treat them. Laboratory based studies with hESC therefore stand to make extraordinary contributions to human health.

Human tumors, and in particular the cancers that affect children, often look like tissues that have not developed normally and whose growth has gone unchecked. In fact, recent studies have shown that, in many cases, tumors arise because genetic mutations in the DNA of normal stem cells lead to disordered development, resulting in formation of malignant rather than normal tissues. For example, leukemia can arise when a mutation occurs in a normal blood stem cell, thus inducing formation of cancerous rather than normal blood. Analogous situations exist in other human tissues and their respective tumors. However, because of the relative rarity of normal stem cells in other parts of the body and our inability to effectively isolate them, very little is yet known about how these stem cells go awry and create cancer. hESC, therefore, represent an invaluable resource for the generation of tissue-specific stem cells and for studies of the genesis of human, and in particular, pediatric cancer.

Several different human cancers are believed to arise either directly or indirectly from stem cells called neural crest stem cells (NCSC). NCSC exist in small numbers throughout the body and contribute to the formation of multiple different tissues including the peripheral nervous system and the pigment cells of our skin. It is our central hypothesis that NCSC-derived tumors arise because genetic mutations in NCSC lead to disordered tissue development and the initiation of cancer.

Ewing's sarcoma family tumors (ESFT) are highly aggressive tumors that primarily affect children and young adults. ESFT have a specific mutation in their DNA and this mutation leads to the creation of a cancer-causing gene. We believe that expression of this abnormal gene in NCSC disrupts normal stem cell differentiation and development and, ultimately, leads to ESFT formation. In this proposal we will use hESC as tools to prove or disprove this theory.

Unfortunately, despite highly toxic and aggressive treatment, the survival rate for patients diagnosed with ESFT remains poor. By creating novel hESC-based models to study the origin and biology of ESFT we aim to gain novel insights into the origin and biology of these tumors that will aid in the development of more effective, less toxic therapies.

Using human embryonic stem cells to treat radiation-induced stem cell loss: Benefits vs cancer risk

University of California, Irvine
Principal Investigator: Charles L. Limoli, Ph.D. Leon J. Thal SEED Grant Program

A variety of stem cells exist in humans throughout life and maintain their ability to divide and change into multiple cell types. Different types of adult derived stem cells occur throughout the body, and reside within specific tissues that serve as a reserve pool of cells that can replenish other cells lost due to aging, disease, trauma, chemotherapy or exposure to ionizing radiation. When conditions occur that lead to the depletion of these adult derived stem cells the recovery of normal tissue is impaired and a variety of complications result. For example, we have demonstrated that when neural stem cells are depleted after whole brain irradiation a subsequent deficit in cognition occurs, and that when muscle stem cells are depleted after leg irradiation an accelerated loss of muscle mass occurs. While an increase in stem cell numbers after depletion has been shown to lead to some functional recovery in the irradiated tissue, such recovery is usually very prolonged and generally suboptimal.

Ionizing radiation is a physical agent that is effective at reducing the number of adult stem cells in nearly all tissues. Normally people are not exposed to doses of radiation that are cause for concern, however, many people are subjected to significant radiation exposures during the course of clinical radiotherapy. While radiotherapy is a front line treatment for many types of cancer, there are often unavoidable side effects associated with the irradiation of normal tissue that can be linked to the depletion of critical stem cell pools. In addition, many of these side effects pose particular threats to pediatric patients undergoing radiotherapy, since children contain more stem cells and suffer higher absolute losses of these cells after irradiation.

Based on the foregoing, we will explore the potential utility and risks associated with using human embryonic stem cells (hESC) in the treatment of certain adverse effects associated with radiation-induced stem cell depletion. Our experiments will directly address whether hESCs can be used to replenish specific populations of stem cells in the brain and muscle depleted after irradiation in efforts to prevent subsequent declines in cognition and muscle mass respectively. In addition to using hESC to hasten the functional recovery of tissue after irradiation, we will also test whether implantation of such unique cells holds unforeseen risks for the development of cancer. Evidence suggests that certain types of stem cells may be prone to cancer, and since little is known regarding this issue with respect to hESC, we feel this critical issue must be addressed. Thus, we will investigate whether hESC implanted into animals develop into tumors over time. The studies proposed here comprise a first step in determining how useful hESCs will be in the treatment of humans exposed to ionizing radiation, as well as many other diseases where adult stem cell depletion might be a concern.

Immunology of neural stem cell fate and function

Stanford University
Principal Investigator: Theo D. Palmer, Ph.D. Comprehensive Research Grant Program

One of the most difficult yet ultimately rewarding goals in stem cell research is to repair damaged neural systems with newly generated neurons. Our work examining neuronal integration and survival in the postnatal and adult brain shows that incoming neurons are uniquely and exquisitely sensitive to the immune response and inflammation that is always present when cells are transplanted into the injured or diseased brain or spinal cord. Here we propose to: 1) further refine our understanding of the molecular mechanisms that promote or inhibit new neuron integration; 2) evaluate pharmacological or biological methods for enhancing transplant efficiency and 3) test the developed techniques in a model of stem cell therapy for treating children who suffer neurological damage due to treatment for brain cancer. Future studies anticipate the use of these interventions to improve stem cell therapies for a variety of neurological injuries and diseases.

FERTILITY AND REPRODUCTION

Very little is known about key events that occur early in pregnancy such as implantation and placental development, yet these events are indispensable for human reproduction. Failure of the placenta to develop properly, or of the very early embryo, the blastocyst, to successfully implant into the wall of the uterus can lead to infertility, miscarriage, problems with the mother's regulation of blood pressure and low birth weight of newborns. The ability of human embryonic stem cells to mature into the specialized cells that form the placenta (trophoblasts) provides the opportunity to gain insight into fertility and pregnancy. In 2007, two awards for research using human embryonic stem cells are approved for funding to understand how trophoblasts form the placenta. This research may lead to the discovery of molecular markers that can be applied to diagnose and treat some forms of infertility and complications that occur early in pregnancy.

LEON J. THAL SEED GRANT PROGRAM

Human Embryonic Stem Cell Differentiation to Trophoblast: Basic Biology and Clinical Translation to Improve Human Fertility
University of California, San Francisco
P.I.: Linda C. Giudice, M.D., Ph.D.
$645,000 for 2 years

Trophoblast differentiation of human ES cells
Burnham Institute for Medical Research
P.I.: Robert G. Oshima, Ph.D.
$750,000 for 2 years

Dollar amount shown is the amount requested rounded to the nearest $5,000, not the amount awarded.

ABSTRACTS

[Provided by applicant]

Human Embryonic Stem Cell Differentiation to Trophoblast: Basic Biology and Clinical Translation to Improve Human Fertility

University of California, San Francisco
Principal Investigator: Linda C. Giudice, M.D., Ph.D. Leon J. Thal SEED Grant Program

In addition to the important potential applications for transplantation and treatment of chronic diseases, human embryonic stem cells (hESC) are also a valuable resource to study early human development relevant to fertility and healthy pregnancies. After fertilization, the human zygote undergoes cell divisions and ultimately becomes the blastocyst that has an inner cell mass and a trophectoderm shell, the precursor of the placenta and the tissue that attaches to the surface of the lining of the uterus, initiating the process of embryonic implantation. After attachment, the placental cells invade into the mother's uterine lining to secure the pregnancy and to establish the placenta for fetal growth and development. Abnormalities in the implantation process can lead to infertility, small babies, and pre-eclampsia that have significant health consequences for women and children. It has been extremely difficult to study the early phases of human implantation because of lack of available tissues (human blastocysts), restrictions on using federal funds for experimentation on human embryos, and the advanced differentiated state (i.e., beyond the trophectoderm stage) of placental cells derived from early terminations of pregnancies. This grant focuses on differentiating hESC to trophectoderm, development of this specialized tissue, and interactions of it with the maternal uterus, as a model of events in the early stages of human implantation.

hESC and human embryos are essential to this project. We propose to study biological processes, biochemical pathways, and key genes expressed during the transition of hESC to trophectoderm and compare them with those of the outer shell isolated from human blastocysts. We shall also identify secreted products from these specialized cells and investigate their effects on human endometrial epithelial cells to get information about how the blastocyst communicates with the maternal uterine lining just as it is about to implant. Finally, we shall identify secreted biomarkers that can be used in future studies as a diagnostic to assess embryo quality in human IVF and as therapies to enhance endometrial receptivity to embryonic implantation in women with implantation-based infertility. This proposal has promise for important translation to improve practical issues in human infertility and pregnancy disorders associated with abnormal embryonic implantation.

Trophoblast differentiation of human ES cells

Burnham Institute for Medical Research
Principal Investigator: Robert G. Oshima, Ph.D. Leon J. Thal SEED Grant Program

Human embryonic stem (ES) cells have the potential to form any cell type, but ironically, the first cell lineage to form during development still represents a surprising challenge.The first cell type to become specialized is an epithelial cell that later defines the boundary between the embryo and mother for the formation of the placenta. The placenta is the key organ that permits the blood of the mother to provide oxygen and nutrients to the fetus. It is composed of multiple cell types that are specialized for different functions but most of the fetal contributions are derived from the trophoblast cell lineage. Nearly 3% of human pregnancies are threatened by deficiencies of the function of the placenta to provide sufficient blood flow. This condition can result in dangerous increases in the mother's blood pressure that threaten the health of both the mother and fetus. Studying the molecular details of the formation and function of the different cell types of the placenta is fundamentally medically important and biologically profound as placental development is a key process that helps defines the human species.

To fully utilize the potential of ES cells, we will start with an understanding of the homogeneity and possible bias in the differentiation fate of available human embryonic stem cell lines. We will characterize multiple human ES cell lines with regard to the types of proteins that form the internal cytoskeleton of the cells. These intermediate filament proteins are widely used for identifying cells of specific tissues. One of these may be characteristic of cells that generate the trophoblast lineage. We will confirm this by comparison with the simultaneous expression of a key determinant of trophoblast cells. To facilitate this analysis we will generate a human ES cell line with a colored marker protein when it changes to the trophoblast lineage. This line will permit the detection and purification of cells choosing this fate.

In mice a trophoblast stem cell has been isolated that is capable of self renewal and retains the capability of from different cell types of the placenta but not the embryo. In mice these cells have been experimentally generated by forced temporary expression of genes capable of triggering the specification of this lineage. However, this has not yet been successful with human ES cells. We propose to isolate trophoblast stem like cells by forcing the expression of genes in human embryonic stem cells that may be expressed at insufficient levels to trigger and maintain the trophoblast stem cell and by inhibiting the late stage differentiation of the same cells. The routine isolation of trophoblast cells from human ES cells will a valuable tool for identifying targets for modulation of placenta formation and function.

HEART DISEASE

Heart disease is the leading cause of death in the United States. Currently there is no cure for severely diseased or damaged hearts except for replacement by transplantation. Donor hearts however are in short supply. Both cardiac muscle cells (cardiomyocytes) and electrically conducting cells (pacemaker cells) contribute to heart health and treatment of heart disease. Recent scientific papers describe promising animal experiments where injection of stem cells appears to improve heart function, yet the injected cells failed to integrate into the sites of damage and to behave like normal heart cells. In 2007, ten awards are approved for funding that focus on developing efficient methods to direct human embryonic stem cells to perform like normal heart muscle or pacemaker cells. The ultimate goals of these research projects are to use heart cells produced from human embryonic stem cells to repair damaged regions of the heart and to integrate and contribute to healing.

LEON J. THAL SEED GRANT PROGRAM

Discovering Potent Molecules with Human ESCs to Treat Heart Disease
Human BioMolecular Research Institute
P.I.: John R. Cashman, Ph.D.
$715,000 for 2 years

Development of Neuro-Coupled Human Embryonic Stem Cell-Derived Cardiac Pacemaker Cells.
Burnham Institute for Medical Research
P.I.: Huei-Sheng Vincent Chen, M.D., Ph.D.
$715,000 for 2 years

Specification of Ventricular Myocyte and Pacemaker Lineages Utilizing Human Embryonic Stem Cells
University of California, San Diego
P.I.: Sylvia M. Evans, Ph.D.
$610,000 for 2 years

Micro Platform for Controlled Cardiac Myocyte Differentiation
University of California, Merced
P.I.: Michelle Khine, Ph.D.
$365,000 for 2 years

Technology for hESC-Derived Cardiomyocyte Differentiation and Optimization of Graft-Host Integration in Adult Myocardium
Stanford University
P.I.: Gregory T. A. Kovacs, M.D., Ph.D.
$635,000 for 2 years

In Vivo Molecular Magnetic Resonance Imaging of Human Embryonic Stem Cells in Murine Model of Myocardial Infarction
Stanford University
P.I.: Phillip Chung-Ming Yang, M.D.
$660,000 for 2 years

Dollar amount shown is the amount requested rounded to the nearest $5,000, not the amount awarded.

COMPREHENSIVE RESEARCH GRANT PROGRAM

Modeling Myocardial Therapy with Human Embryonic Stem Cells
University of California, San Francisco
P.I.: Harold S. Bernstein, M.D., Ph.D.
$2,230,000 for 4 years

Embryonic Stem Cell-Derived Therapies Targeting Cardiac Ischemic Disease
University of California, San Francisco
P.I.: Randall James Lee, M.D., Ph.D.
$2,525,000 for 4 years

Chemical Genetic Approach to Production of hESC-derived Cardiomyocytes
Burnham Institute for Medical Research
P.I.: Mark Mercola, Ph.D.
$3,040,000 for 4 years

Engineering a Cardiovascular Tissue Graft from Human Embryonic Stem Cells
Stanford University
P.I.: Christopher K. Zarins, M.D.
$2,620,000 for 4 years

Dollar amount shown is the amount requested rounded to the nearest $5,000, not the amount awarded.

ABSTRACTS

[Provided by applicant]

Discovering Potent Molecules with Human ESCs to Treat Heart Disease

Human BioMolecular Research Institute
Principal Investigator: John R. Cashman, Ph.D. Leon J. Thal SEED Grant Program

This work is directly relevant to human embryonic stem cell (hESC) research because it brings new ideas about novel compounds to affect cardiomyogenesis. The work addresses an urgent need to develop new agents to treat cardiovascular disease. We will develop potent and selective drug-like molecules as cardiomyocyte differentiation agents.

Heart disease is the leading cause of mortality and decline in the quality of life in the developed world. The ability of hESCs to form cardiomyocytes has spawned hope that these cells may be used to replace damaged myocardium. Despite their ability to form cardiomyocytes, efficient and controlled cardiomyogenesis in ESC cultures has not been achieved due to the unavailability of differentiation agents and an incomplete understanding of the pathways that regulate cardiac development.

Success has been achieved in developing a robust and dependable high-throughput assay to study the effects of small molecules on cardiomyocyte differentiation. Powerful cell-based assays were developed and provided readouts that led to high-content results because multiple signals were probed. The assay is capable of capturing fast or long-acting biology because of the time-course readouts. Cell-based assays are superior to molecular screens because the cell-based assay delivers active compounds or 'hits' that are permeable and non-cytotoxic. Moreover, refined 'hits' can be used as probes to reveal novel signaling pathways and proteins that control differentiation, in a process termed chemical biology. By taking advantage of knowledge of the current 'hits' we will rapidly synthesize novel drug-like compounds in a low-risk approach to. The 'hits' will be refined and improved through an efficient synthetic process we use in our lab called 'Dynamic Medicinal Chemistry'.

Even after miniaturization and automation, screening is still expensive. A key to improve the screening process is to use pharmacologically active, drug-like compounds to provide rich target-relevant information. Intelligently designing libraries for screening by incorporating drug-like features into 'lead' library design will improve the attrition rate and lead to more pharmacologically relevant compounds for future studies.

This proposal is directly responsive to the California Institute for Regenerative Medicine SEED Grant Program because it provides for developing and testing new agents of use in cardiomyoenesis of hESCs. Importantly, it brings new investigators and a collaborative approach to the stem cell field. The agents discovered and developed may hold great promise as the groundwork for future medications development for a new class of damaged myocardium replacement agents. The theoretical rationale for the work is the use of high-content screening coupled with drug-like new agent discovery approaches. The work will also be of use in the elucidation of key biochemical targets and novel signaling pathways important in hESC cardiomyogenesis.

Development of Neuro-Coupled Human Embryonic Stem Cell-Derived Cardiac Pacemaker Cells.

Burnham Institute for Medical Research
Principal Investigator: Huei-Sheng Vincent Chen, M.D., Ph.D. Leon J. Thal SEED Program

Optimal cardiac function depends on the properly coordinated cardiac conduction system (CCS). The CCS is a group of specialized cells responsible for generating cardiac rhythm and conducting these signals efficiently to working myocardium. This specialized CCS includes the sinoatrial node, atrioventricular node and His-Purkinje system. These specialized pacemaking /conducting cells have different properties from the surrounding myocytes responsible for the contractile force. Genetic defects or postnatal damage by diseases or aging processes of these cells would result in impaired pulse generation (sinus node dysfunction, SND) or propagation (heart block). Implantation of an electronic cardiac pacemaker is necessary for intolerant bradycardia to restore cardiac rhythm. However, the electronic implantable pacemaker has multiple associated risks (e.g. infections) and requires frequent generator changes due to limited battery life. Sinus node dysfunction is a generalized abnormality of cardiac impulse formation and accounts for >30 percent of permanent pacemaker placements in the US. A perfect therapy to SND will be to repair or replace the defective sinus node by cellular or genetic approaches. Many recent studies have demonstrated, in a proof-of-concept style, of generating a biological pacemaker by implanting various types of progenitor or stem cells into ventricular myocardium to form a pulse-generating focus. However, a perfect biological pacemaker will require good connections with the intrinsic neuronal network for proper physiological responses. Elucidation of the factors controlling the evolution of pacemaker cells and their interaction with the peripheral neuronal precursor cells (neural crest cells, NCCs) will be paramount for creating an adaptive biological pacemaker. The NCCs have been shown to be contiguous with the developing conduction system in embryonic hearts of humans. However, the influence and interaction of the NCCs with the developing cardiac pacemaker cells remains unclear. In addition, there is no simple marker for identifying the pacemaker cells and the electrophysiological (EP) recording is the only physiological method to trace the evolution of cardiac pacemaker cells from human embryonic stem cells (hESCs). We have successfully obtained the EP properties of early hESC-derived cardiomyocytes. We propose here an in vitro co-culture system to study fate of the pacemaker cells evolved from hESCs and to investigate the influence of NCCs on the early, cardiac committed myocytes derived from hESCs. Such a study will provide insight in the development of pacemaker cells and in the mechanisms of early neuro-cardiac interaction. Results from the proposed study may suggest strategies for developing efficient and neuro-coupled cardiac pacemakers from ESCs. These neuro-coupled biological pacemaker cells may one day used clinically to replace the need for implanting an electronic pacemaker for the treatment of intolerant bradycardia.

Specification of Ventricular Myocyte and Pacemaker Lineages Utilizing Human Embryonic Stem Cells

University of California, San Diego
Principal Investigator: Sylvia M. Evans, Ph.D. Leon J. Thal SEED Grant Program

Heart failure is a leading cause of mortality in California and the United States. Currently, there are no "cures" for heart failure.Other life threatening forms of heart disease include dysfunction of cardiac pacemaker cells, necessitating implantation of mechanical pacemakers. Although mechanical pacemakers can be efficacious, there are potential associated problems, including infection, limited battery half-life, and lack of responsiveness to normal biological cues.
Our research with human embryonic stem cells will be aimed at developing therapies for heart failure, and cardiac pacemaker dysfunction. In each of these disease settings, one might effect a "cure" by replacing worn out or dysfunctional cardiac cells with new ones. In the case of heart failure, the cells that need to be replaced are heart muscle cells, which do the majority of the work in the heart. In the case of pacemaker dysfunction, the cells that need to be replaced are pacemaker cells, a highly specialized type

of heart muscle cell. To replace these cells, we need to find cells that can become heart muscle or cardiac pacemaker cells, understand how to generate fairly large numbers of them, and how to persuade them to become either heart muscle or cardiac pacemaker cells. Potential cardiac progenitor cells may come from a number of different sources, either from patients themselves, or from extrinsic sources. Regardless of the source,we need to define factors which will make the cells multiply and will make them become the cell type that we need for repair.

The biology of human heart cells is likely to be distinctive from that of heart cells from other animals. For example, a human heart has to function for multiple decades, unlike hearts of other animals who live in general for shorter periods of time. The size, required function, and rhythm of the human heart are also distinct from that of other animals. For these reasons, for repair of human heart, it is important to study human cardiac progenitors and to define pathways required to grow them and to differentiate them utilizing human cells as a model experimental system.

Our proposed research will utilize human embryonic stem cells as a source of cardiac progenitors. As human embryonic stem cells can turn into many different kinds of cells, we will create special lines of human embryonic stem cells that will become fluorescent when they adopt the cardiac progenitor, heart muscle, or pacemaker state. These lines will then be treated with a large number of small molecules to find small molecules which amplify cells the number of fluorescent cells in each of these states. The small molecules activate known biochemical pathways, so we can then use the small molecules themselves, or activate identified pathways to achieve the goal of obtaining sufficient numbers of specific cardiac cell types for cardiac therapy.

Micro Platform for Controlled Cardiac Myocyte Differentiation

University of California, Merced

Principal Investigator: Michelle Khine, Ph.D. Leon J. Thal SEED Grant Program

Congestive heart failure, the inability of the heart to continue to pump effectively due to damage of its muscle cells, affects approximately 4.8 million Americans and is a leading cause of mortality. Causes of the irreversible damage to the cardiomyocytes that results in congestive heart failure include hypertension, heart attacks, and coronary disease. Because the cadiomyocytes in the adult heart tissue are terminally differentiated and thus cannot regenerate themselves, once they are damaged, they are irreversibly damaged. As a consequence, despite the advances in medical devices and pharmaceuticals, still more than 50% of congestive heart failure patients die within 5 years of initial diagnosis.

The goal therefore must be to restore the heart cells' functions. This is possible by transplanting fetal and neonatal cardiomyocytes which can then integrate into the host tissue. This approach has demonstrated success in improving heart function. However, the limited availability of fetal donors has prevented its adoption as a viable therapeutic approach.

Embryonic stem cells can overcome this challenge as they proliferate continuously in vitro and can be furthermore stimulated to differentiate. Embryoid bodies are three-dimensional clusters of heterogenous stem cells, some of which contain cardiac myocytes, which demonstrate characteristic spontaneous contractions. Controlled and efficient differentiation of the stem cells into cardiomyocytes and an effective way to characterize/verify these cells is critical. Ensuring a pure population of cardiac myocytes is essential because otherwise there is a high-likelihood of tumor formation when transplanted. Previous studies have shown that a low percentage of all embryoid bodies spontaneously form cardiomyocytes.

Our goal is to therefore develop a self-contained system to grow and controllably differentiate the human embryonic stem cells into cardiomyocytes in high-yields. Few studies have characterized the types of cardiac myocytes in the differentiating human EBs. Our strategy is to use electrical and chemical cues to

induce the high-yield differentiation of stem cells into cardiomyocytes and to monitor this process over time both electrically and optically.

Technology for hESC-Derived Cardiomyocyte Differentiation and Optimization of Graft-Host Integration in Adult Myocardium

Stanford University

Principal Investigator: Gregory T.A. Kovacs, M.D., Ph.D. Leon J. Thal SEED Grant Program

Stem cells therapies hold great promise in the treatment of cardiac diseases such as coronary heart disease or congestive heart failure. Thanks to their ability to transform into almost any kind of tissue, engrafted stem cells can potentially replace damaged heart tissues with healthy tissues, effectively restoring the heart's original functions. While initial studies demonstrated the potential benefits of stem cell injection for repairing heart damage, they told researchers little about exactly how improvements were made to the heart and how the improvement might be enhanced. Also, there is concern that the stem cells could negatively impact some aspects of heart function and lead to disturbances of heart rhythm and future attacks.

In light of this, we propose to develop a model to study the detailed interaction of stem cells and healthy heart tissue in the laboratory, where events within the cells and between the cells can be measured accurately and many experiments can be done to increase our understanding, without the use of human subjects. Specifically, we plan to focus on two main goals.

The first goal is to develop a platform to better understand the gradual transition that stem cell lines make as they mature into heart cells, process known as differentiation. We will record the electrical activity arising from newly formed heart cells to determine when exactly they form and how the behave in response to electrical stimuli or drugs as they mature. This will tell us more about the behavior of the cells that could be injected into the heart so that we know how they will respond when they merge with the heart and when is the best time to introduce them.

The second goal, building on the first one, is to observe how the stem cells make contact with the heart cells, including how they grow together mechanically and how they begin to communicate electrically as a repaired tissue. This will be carried out by growing the stem cells and heart cells separately and then allowing them to grow together, just as they would in the heart. Simultaneous recording of electrical activity at numerous locations in the culture will let us map the activity across the culture and evaluate the communication between heart cells (host) and stem cells (graft).

Understanding the microscopic nature of integration of stem cells into healthy tissue will lead to a greater knowledge of what can happen when stem cells are injected into the heart and begin to replace the non-functional tissue and connect to healthy tissue. Insights gained with such model should lead to a better understanding of the repair process and highlight strategies for making stem cell-based therapies safer and more effective. This model will also allow testing and development of chemical or electrical manipulations that would increase the yield and reliability of the differentiation process, paving the way for the ultimate scale-up of stem cell therapies for clinical use.

In Vivo Molecular Magnetic Resonance Imaging of Human Embryonic Stem Cells in Murine Model of Myocardial Infarction

Stanford University

Principal Investigator: Phillip Chung-Ming Yang, M.D. Leon J. Thal SEED Grant Program

Magnetic resonance imaging (MRI) has emerged as one of the predominant modalities to evaluate the effects of stem cells in restoring the injured myocardium. However, MRI does not enable assessment of a fundamental issue in cell therapy, survival of the transplanted cells. The transplanted human embryonic cells (hESC) must at the very least survive to restore the injured myocardium. This research proposal will address this specific challenge to image non-invasively both the survival of the transplanted hESC and the resultant restoration of the myocardium through sensitive detection of the molecular events indicating hESC survival and rapid imaging of myocardial function. In order to achieve this dual capability, there are 2 primary considerations: 1) amplification of molecular signals and 2) high spatial and temporal resolution imaging of the myocardium.

No single imaging modality will fulfill all needs of non-invasive molecular imaging in the heart. Only an imaging modality that optimizes the 2 technical specifications will provide physiologically relevant meaning of the molecular signal of the transplanted hESC. The molecular signal will be useful if some correlation between hESC survival and functional restoration can be established. In order to address these critical issues, this proposal will describe efforts to implement molecular MRI to image both the survival of transplanted hESC and restoration of cardiac function using mouse model of myocardial infarction.

This research proposes an integrated, multidisciplinary approach to converge innovative approaches in MRI and stem cell biology to address a fundamental yet very critical issue in cardiac restoration: survival of hESC following transplantation into the injured myocardium. This proposal combines novel molecular techniques with the high resolution capabilities of MRI. Upon conclusion of this research, an integrated MRI platform will be developed to allow dual evaluation of the survival of transplanted hESC and their effects on myocardial function. Maturation of this imaging technology will ultimately enable accurate assessment of the survival of hESC and restoration of recipient tissue in all human organs.

Modeling Myocardial Therapy with Human Embryonic Stem Cells

University of California, San Francisco

Principal Investigator: Harold S. Bernstein, M.D., Ph.D. Comprehensive Research Program

Five million people in the U.S. suffer with heart failure, at a cost of $30 billion/year. Heart failure occurs when the heart is damaged and becomes unable to meet the demands placed on it. Unlike some tissues, heart muscle does not regenerate. Human embryonic stem cells grow and divide indefinitely while maintaining the potential to develop into many tissues of the body, including heart muscle. They provide an unprecedented opportunity to both study human heart muscle in culture in the laboratory, and advance cell-based therapy for damaged heart muscle. We have developed methods for identifying and isolating specific types of human embryonic stem cells, stimulating them to become human heart muscle cells, and delivering these into the hearts of mice that have had a heart attack. This research will identify those human embryonic stem cells that are best at repairing damaged heart muscle, thereby treating or avoiding heart failure.

Embryonic Stem Cell-Derived Therapies Targeting Cardiac Ischemic Disease
University of California, San Francisco
Principal Investigator: Randall James Lee, M.D., Ph.D. Comprehensive Research Program

Cardiovascular disease (CVD) is the leading cause of death in the United States. Over one million Americans will suffer from a new or recurrent heart attacks this year and over 40 percent of those will die suddenly. In addition, about two-thirds of the patients develop congestive heart failure; and in people diagnosed with CHF, sudden cardiac death occurs at 6-9 times the general population rate. Heart transplantation remains the only viable solution for severely injured hearts; however, this treatment is limited by the availability of donor hearts. Therefore, alternative strategies to treat end stage heart failure and blocked blood vessels are needed. The objective of this proposal is to determine whether human embryonic stem (hES) cell can be used for repairing the heart. Our collaborator Advanced Cell Technology (ACT) has recently succeeded in identifying conditions for the reproducible isolation of hES cells which have the characteristics of cells which form blood vessels and heart muscle. This proposal will assess whether the hES cells can form new functional blood vessels and repair injured heart muscle in a rat model of heart attacks. Results from these studies will help develop new therapies for treating patients with heart attacks.

Chemical Genetic Approach to Production of hESC-derived Cardiomyocytes
Burnham Institute for Medical Research
Principal Investigator: Mark Mercola, Ph.D. Comprehensive Research Grant Program

Adult heart muscle cells retain negligible proliferative capacity and this underlies the inability of the heart to replace muscle cells that are lost to injury, such as infarct, and underlies progression to heart failure. To date, no stem cell therapiy has produced significant cardiomyocyte replacement. Instead, transplanted cells, if they persist at all, produce endothelial cells or fibroblasts and the ameliorating effects on heart function that have been reported have been achieved by improving contractility, perfusion or other processes that are impaired in the failing heart. This proposal is to develop specific reagents and ultimately drugs to stimulate regeneration. Our approach integrates advances in stem cell biology, high-throughput (HT) biology, informatics and proteomics to identify small molecules, proteins and signal transduction pathways that control heart muscle formation from human embryonic stem cells (hESCs). High throughput assays will be developed and implemented to identify genes, signaling proteins, and small molecules that that control important steps in the differentiation, proliferation, and maturation of cardiac cells. Computer modeling and informatics will be used to identify and validate the signaling pathways that direct these critical processes. The discovery of small molecules and pathways will lead to protocols for 1) efficient directed differentiation of cardiomyogenic precursors from hESCs for research into transplantation and toxicology, 2) biotech reagents to stimulate cardiomyocyte renewal through directed differentiation of hESCs, and 3) cellular targets or lead compounds to develop drugs that stimulate regeneration from endogenous cells.

Engineering a Cardiovascular Tissue Graft from Human Embryonic Stem Cells
Stanford University
Principal Investigator: Christopher K. Zarins, M.D. Comprehensive Research Grant Program

Cardiovascular disease (CVD) affects more than 71 million Americans and 1.7 million Californians. Recently, engineered cardiovascular tissue grafts, or "patches", including one made from mouse embryonic stem cells (ESC), have shown promising results as a future therapy for CVD. Our overall goal is to extend these recent results to human ESC as follows.

Aim 1: Apply mechanical stretch and electrical pacemaker-like stimulation to hESC-derived heart cells in order to make them stronger and beat at the same time.

Current methods to turn hESC into heart cells do not result in the organization required to generate enough strength to support a weak heart and to avoid irregular heart beats. We will use specially engineered devices to apply mechanical stretch and electrical pacemaker-like stimulation to hESC-derived heart cells in order to strengthen them and make them beat in unison.

Aim 2: Engineer a cardiovascular patch from hESC-derived heart cells in order to make a potential new therapy for heart disease.

Recently, heart cells from mouse ESC, supporting structures called scaffolds, and mechanical stretch have successfully been combined to engineer a cardiovascular patch. We will combine the hESC-derived heart cells from Aim 1, scaffolds, and the same stretch and pacemaker-like stimulation as in Aim 1 to engineer a cardiovascular patch. In addition, we will add a specialized substance called VEGF to our patch so that, potentially, a blood supply will form around it after it is implanted on a diseased heart. We believe a blood supply will be necessary to keep our patch healthy, and in turn, this will allow our patch to help a damaged heart pump better.

Aim 3: Assess whether our patch can remain healthy and also strengthen the heart of a rat after it has undergone a heart attack.

We will first implant our cardiovascular patch in the rat aorta, the main blood vessel that supplies blood to the body, to test whether the patch remains healthy and whether it can contract and beat on its own. We will first use the aortic position because we feel it will allow us to assess the inherent function of the patch, thus facilitating our efforts to improve its design. After testing in the aortic position, we will implant the patch over damaged heart tissue in a rat that has undergone an experimentally created heart attack. Over a period of several weeks, we will use novel imaging techniques, ultrasonography, echocardiography, and electrocardiography to non-invasively test whether the patch remains healthy and whether the patch helps the damaged heart pump better.

We believe the above aims will address questions relevant to hESC-based cardiovascular therapies and will provide vital information needed for safe and effective future clinical translation. As we will evaluate both federally and non-federally approved cell lines, and thus unlikely to receive federal funding, we will need to rely on the support provided by CIRM to carry out our objectives.

METABOLIC DISEASES AND CHILDHOOD DISORDERS

Many inherited and acquired diseases have a metabolic component that first arises during childhood. Some diseases are serious enough that the affected individuals die young, while other diseases can cause substantial disability and suffering throughout life. In 2007, six awards are approved for funding in the category of Metabolic Diseases and Childhood Disorders (in addition to awards supported in other disease categories in which the diseases can adversely affect children as well as adults, such as Heart Disease, Cancer, and Injury to the Nervous System).

AUTISM

LEON J. THAL SEED GRANT PROGRAM

MicroRNAs in Human Stem Cell Differentiation and Mental Disorders
The J. David Gladstone Institutes
P.I.: Fen-Biao Gao, Ph.D.
$795,000 for 2 years

DIABETES

LEON J. THAL SEED GRANT PROGRAM

Endodermal differentiation of human ES cells
University of California, San Francisco
P.I.: Didier Y.R. Stainier, Ph.D.
$640,000 for 2 years

Dollar amount shown is the amount requested rounded to the nearest $5,000, not the amount awarded.

LIVER DISEASE

COMPREHENSIVE RESEARCH GRANT PROGRAM

An in vitro and in vivo comparison among three different human hepatic stem cell populations.
University of California, Davis
P.I.: Mark Allen Zern, M.D.
$2,505,000 for 4 years

MUSCULAR DYSTROPHY

LEON J. THAL SEED GRANT PROGRAM

Derivation and characterization of human ES cells from FSHD embryos
University of California, Irvine
P.I.: Kyoko Yokomori, Ph.D.
$635,000 for 2 years

RENAL DISEASE

COMPREHENSIVE RESEARCH GRANT PROGRAM

Preclinical Model for Labeling, Transplant, and In Vivo Imaging of Differentiated Human Embryonic Stem Cells
University of California, Davis
P.I.: Alice F. Tarantal, Ph.D.
$2,260,000 for 4 years

INTESTINAL DISEASE

LEON J. THAL SEED GRANT PROGRAM

Differentiation of Human Embryonic Stem Cells to Intestinal Fates
Stanford University
P.I.: Calvin Jay Kuo, M.D., Ph.D.
$580,000 for 2 years

Dollar amount shown is the amount requested rounded to the nearest $5,000, not the amount awarded.

ABSTRACTS

[Provided by applicant]

AUTISM

MicroRNAs in Human Stem Cell Differentiation and Mental Disorders

The J. David Gladstone Institutes
Principal Investigator: Fen-Biao Gao, Ph.D. Leon J. Thal SEED Grant Program

Many mental disorders are closely associated with problems that occur during brain development in early life. For instance, by 2 years of age, autistic children have larger brains than normal kids, likely due to, at least in part, excess production of neurons and support cells, the building blocks of the nervous system. In autistic brains, how neurons grow various thread-like processes also shows some abnormalities. The cause of autism is complex and likely involves many genetic factors. These developmental defects are also associated with mental disorders caused by single-gene mutations, such as Rett syndrome and fragile X syndrome, the most common form of inherited mental retardation, whose clinical features overlap with autism. However, what causes the developmental defects in brains of children with different mental disorders is largely unknown.
In recent years, an exciting new regulatory pathway was discovered that may well contribute to the etiology of mental disorders. The major player in this novel pathway is a class of tiny molecules 21-22 nucleotides long, called microRNAs. Through a unique mechanism, these small molecules control gene expression during the development of many organs, including the brain through a unique mechanism. Their importance in mental disorders is underscored by the discovery that they are somehow associated with FMR1, the gene that is mutated in fragile X syndrome.

In this application, we will study the roles of microRNAs in human embryonic stem cell maintenance and differentiation into neurons. The activities of some microRNAs will be manipulated in stem cells or differentiated neurons and their effects will be accessed. These studies will help us further understand the biology of human embryonic stem cells, and how they cells can be controlled to differentiate into desirable neuronal cells once implanted into human central nervous system. Those fundamental knowledges are essential for the future development of stem cell therapies for a wide range of mental disorders and age-dependent neurodegenerative diseases.

DIABETES

Endodermal differentiation of human ES cells

University of California, San Francisco
Principal Investigator: Didier Y.R. Stainier, Ph.D. Leon J. Thal SEED Grant Program

The goals of this proposal are to investigate endodermal differentiation and proliferation in human ES cell cultures. Endodermal cells give rise to the epithelial lining of the respiratory and digestive tract as well as to the liver and pancreas. The future treatment of diseases such as type I diabetes using stem cell therapy relies on our ability to differentiate stem cells into endoderm, a prerequisite step to forming pancreatic beta cells. In 2005, D'Amour et al. reported the efficient differentiation of human ES cells into endoderm. This report provides a potentially effective protocol that needs to be further evaluated (specific aim 1). In addition, given that the success of stem-cell therapy depends on our ability to generate large numbers of differentiated cells (e.g. 200-700 million beta cells per patient are currently being used in the Edmonton protocol), we will investigate the ability of the endodermal generated in specific aim 1 cells to proliferate in culture (specific aim 2).

LIVER DISEASE

An in vitro and in vivo comparison among three different human hepatic stem cell populations.

University of California, Davis
Principal Investigator: Mark Allen Zern, M.D. Comprehensive Research Grant Program

Because there is still considerable morbidity and mortality associated with the process of transplantation, and because more than a thousand people die each year while on the liver transplantation list, it is evident that improved and safer liver transplantation would be valuable, as would approaches that provide for an increased number of transplantations in a timely manner. A technology that might address these issues is the development of a human liver cell line that can be employed in liver cell transplantation or in a bioartificial assist device. Developing such a cell line from human embryonic stem cells (hESC) or from other human stem cell sources would provide a valuable tool for pharmacology studies, as well as for use in cell-based therapeutics.

In the proposed studies, we will differentiate human embryonic stem cells or fetal liver cells or bone-marrow derived cells so that they act like liver cells in culture. Once it has been established that the cells are acting like liver cells by producing normal human liver proteins, and that they do not act like cancer cells, the cells will be injected into the livers of immunoincompetent mice that do not rejects human cells. Then we will evaluate whether the cells grow and thrive in the mouse livers, whether they still produce high levels of human liver-specific proteins, whether they produce tumors in the mouse livers, and whether they can replace damaged mouse liver cells with human cells. One of the ways this will be done is to label the cells with a marker gene and to image the marker gene in the livers of the mice with special x-ray machines that can distinguish a few hundred human cells in the mouse liver. Finally, we will infuse the human liver stem cells into the liver of monkeys to determine if they will grow in the monkey livers, because the monkeys are more similar to man. Such studies should be done in nonhuman primates before clinical studies are undertaken to employ these cells to replace abnormal liver cells in man. Our intent is these studies is to compare and contrast three types of stem cells to determine which will be the most effective cells to use in human studies.

If the studies are successfully undertaken, we will establish a clinically useful and safe liver cell line that could be used to repopulate an injured liver in a safer and less expensive manner than with liver transplantation; moreover, all people who had liver failure or an inherited liver disease could be treated, because there would be an unlimited supply of liver cells.

MUSCULAR DYSTROPHY

Derivation and characterization of human ES cells from FSHD embryos

University of California, Irvine
Principal Investigator: Kyoko Yokomori, Ph.D. Leon J. Thal SEED Grant Program

Facioscapulohumeral muscular dystrophy (FSHD) is the third most common hereditary muscular dystrophy. It is autosomal dominant, meaning that if one of the parents has the disease, their children have a 50:50 chance of getting it, too. FSHD is characterized by progressive weakness and atrophy of facial, shoulder and upper arm musculature, which can spread to other parts of the body. In some cases, it is accompanied by hearing loss and, in severe cases, mental retardation. There is no cure or treatment of this disease since the gene(s) responsible for this disease has not been identified. One thing that is clear is that the majority of FSHD is linked to a decrease in the number of repeats of a DNA sequence called D4Z4 located at the end of chromosome 4. When shortening of this repeat region occurs in either chromosome 4, the person gets FSHD. However, it is unclear how shortening of this repeat leads to the disease. We found that this D4Z4 repeat cluster contains 'heterochromatin' structure, which is associated with gene silencing. This heterochromatin structure includes specific methylation of histone H3 and the recruitment of heterochromatin binding proteins HP1 and cohesin. HP1 is known to be required for gene silencing. Importantly, we found that this heterochromatin structure is uniquely lost in FSHD patient cells. Surprisingly, the minor population of FSHD patients who do not exhibit any repeat shortening also lack this heterochromatin structure in the D4Z4 repeat on chromosome 4. Therefore, FSHD is a 'heterochromatin abnormality' disease, in which loss of heterochromatin at D4Z4 repeats leads to disease manifestation. We hypothesize that the normal heterochromatin structure spreads silencing effects on to other genes, but in FSHD this effect is lost and these genes that are normally silent may be abnormally expressed. Since we found that this heterochromatin structure is already established in embryonic stem (ES) cells under normal circumstances, it is of vital importance to examine this process in FSHD ES cells. This would be important to understand how heterochromatin establishment is compromised during development and, as a result, which genes are affected. However, no FSHD ES cells are currently available. Generous and courageous families with FSHD in their history donated in vitro fertilized embryos for research use in the hope of improving the life of FSHD patients in the future. Therefore, our major goal is to establish FSHD ES cell lines not only for our research, but also for use in the FSHD research community. We hope to optimize a protocol to differentiate these cells into skeletal muscle cells for a comparative analysis between normal and FSHD ES cells during development. I believe that the proposed project will make significant contributions to understanding the etiology and pathogenesis of FSHD as well as to possibly develop therapeutic strategies to improve the physical functioning of FSHD patients.

RENAL DISEASE

Preclinical Model for Labeling, Transplant, and In Vivo Imaging of Differentiated Human Embryonic Stem Cells

University of California, Davis
Principal Investigator: Alice F. Tarantal, Ph.D. Comprehensive Research Grant Program

The derivation and culture of human embryonic stem cells has provided new possibilities for treatment of a wide variety of human diseases because these cells have the potential to help regenerate and repair many types of damaged tissue. Diseases for which such cell-based treatments may be helpful include obstructive renal disease, a disorder for which there has been little progress made in terms of treatment. Infants with this and other inherited kidney disease may be severely compromised before birth and treatments necessary to prolong their life may be accompanied by severe side effects. This raises many

difficulties not only for these young patients but also for their families. If new ways to treat these infants prior to birth can be developed, this could lead to the delivery of healthy babies at full term. The use of cells obtained from human embryonic stem cells to repair and treat damaged kidneys prior to birth offers promise to improve survival and quality of life for these babies. Since it is clear that embryonic stem cells have vast potential to form a variety of cell types, it is possible that the kinds of cells needed to provide repair could be obtained and treatments initiated prior to birth. The studies proposed will assess ways to obtain such cells and the effectiveness of such treatments. Ultimately, even small improvements in function of damaged kidneys following embryonic stem cell-based therapies may increase survival and eliminate the need for dialysis or kidney transplants. Although methods to grow embryonic stem cells and even obtain cells that could be useful for treating some human diseases have been described, the use of these cells for human therapies remains highly controversial because their safety remains untested. While these cells have great potential and promise to form cell types useful for treatment of disease, they also have the potential for uncontrolled growth and to form tissues that would be harmful. Therefore, studies must be performed and techniques must be developed to carefully examine the use of these cells in relevant models of human disease, and before they are ever considered for human treatments. The overall intent of these studies is to develop techniques that can be used to test the safety of human embryonic stem cell-based therapies, and to determine ways to evaluate the cells after they have been injected into the body. As we develop new treatments for obstructive kidney disease, we will use this model system to explore these essential safety questions related to stem cell therapies. The studies proposed will fill a critical need for new treatments for kidney disease, ways to monitor cells in patients, and develop methods to assess safety issues associated with the transfer of this research to human patients.

INTESTINAL DISEASE

Differentiation of Human Embryonic Stem Cells to Intestinal Fates

Stanford University

Principal Investigator: Calvin Jay Kuo, M.D., Ph.D. Leon J. Thal SEED Grant Program

The roughly 25 feet of intestine in the adult human play numerous essential roles in daily life, such as nutrient absorption, secretion of hormones, and serving as a barrier to infection. Commensurate with these diverse roles, diseases of the intestine are a considerable source of human morbidity and mortality. Indeed, numerous pathologic conditions including inflammatory bowel diseases, mesenteric ischemia, congenital syndromes and trauma, with or without concomitant intestinal resection, all impair intestinal function to the extent that 'short-gut' syndromes develop-- resulting in effective intestinal failure. Current therapies rely on supportive measures such as total parenteral nutrition, in which patients receive all of their nutrition intravenously, or even intestinal transplantation.

The adult intestine is populated by specialized but highly active intestinal stem cells, which ideally could be harnessed for stem cell therapies of these disabling conditions. However, despite intensive research, no methods currently exist for identifying, isolating, and growing these intestinal stem cells for therapeutic purposes.

Our goal is to develop technologies enabling human embryonic stem (hES) cells to be reliably converted to intestinal cells in culture. Human ES cells can be readily grown in culture but represent a completely undifferentiated tabula rasa. Here, we propose studies to convert hES cells to intestinal stem cells and thence to mature intestinal cells. Towards this goal, we have developed the first methodology to induce intestinal cells to divide and expand as cultures, or 'explants' outside of the body. This success has been reliant on the provision in our explants of a nutritive 'niche', a specialized area in which signals conductive to intestinal stem cell survival are highly concentrated. In this proposal, the hES cells will be placed in this niche of our explant culture, amidst signals that would promote their conversion from a na?ve state

into intestinal stem cells and their mature progeny. We will further refine these methods by coaxing hES cells along the first steps towards intestine prior to placing them in the explant niche, as well as by adding hormones to encourage growth of intestinal cells. The use of hES cells could greatly enhance the growth of our explant cultures, vastly expanding the yield of cultured intestine.

The therapeutic applications of this work are clear. The combination of ES cell technology along with our explant culture system holds considerable promise for the eventual generation of large quantities of intestinal stem cells, or even artificial intestine. Hopefully, these will yield effective therapies for the numerous conditions resulting in effective intestinal failure, for which currently available therapies are decidedly suboptimal.

DISEASES OF THE NERVOUS SYSTEM

Degenerative diseases can strike different cell populations and locations in the nervous system with devastating consequences. While various neural degenerative diseases can affect very different cell types, human embryonic stem cells have the potential to differentiate into the desired neural phenotypes to replace the missing or defective cells and restore lost function. The list of candidates for cell replacement therapy include forebrain neurons for Alzheimer's Disease; oligodendrocytes for Multiple Sclerosis; dopaminergic neurons for Parkinson's Disease; motor neurons for Amyotrophic Lateral Sclerosis (ALS, also known as Lou Gehrig's Disease) and Spinal Muscular Atrophy (SMA); hair cells for deafness and retinal pigmented epithelial cells for some types of blindness. Thus developing reliable methods to generate the needed cells for replacement is a major goal for regenerative medicine. Substantive progress also can be made by using human embryonic stem cells to produce cellular models of the disease that then can be applied to drug discovery and to study how the disease progresses. In 2007, 20 projects were approved for funding that focus on approaches toward treatments and cures for degenerative diseases of the nervous system.

ALZHEIMER'S DISEASE

LEON J. THAL SEED GRANT PROGRAM

Generation of forebrain neurons from human embryonic stem cells
University of California, San Diego
P.I.: Anirvan Ghosh, Ph.D.
$615,000 for 2 years

Development of human ES cell lines as a model system for Alzheimer disease drug discovery
University of California, Irvine
P.I.: Frank M. LaFerla, Ph.D.
$495,000 for 2 years

COMPREHENSIVE RESEARCH GRANT PROGRAM

Using Human Embryonic Stem Cells to Understand and to Develop New Therapies for Alzheimer's Disease
University of California, San Diego
P.I.: Lawrence S. B. Goldstein, Ph.D.
$2,515,000 for 4 years

Dollar amount shown is the amount requested rounded to the nearest $5,000, not the amount awarded.

AMYOTROPHIC LATERAL SCLEROSIS (ALS) /SPINAL MUSCULAR ATROPHY (SMA)

LEON J. THAL SEED GRANT PROGRAM

In vitro differentiation of hESCs into corticospinal motor neurons
University of California, Santa Cruz
P.I.: Bin Chen, Ph.D.
$500,000 for 2 years

Retinoic Acid-FGF Antagonism during Motor Neuron Differentiation of Human ES Cells
Burnham Institute for Medical Research
P.I.: Gregg Duester, Ph.D.
$760,000 for 2 years

Gene regulatory mechanisms that control spinal neuron differentiation from hES cells.
The Salk Institute for Biological Studies
P.I.: Samuel L. Pfaff, Ph.D.
$810,000 for 2 years

Genetic manipulation of human embryonic stem cells and its application in studying CNS development and repair
University of California, San Diego
P.I.: Binhai Zheng, Ph.D.
$645,000 for 2 years
Also referenced in: Spinal Cord Injury

COMPREHENSIVE RESEARCH GRANT PROGRAM

Molecular and Cellular Transitions from ES Cells to Mature Functioning Human Neurons
The Salk Institute for Biological Studies
P.I.: Fred H.Gage, Ph.D.
$2,880,000 for 4 years

hESC-Derived Motor Neurons For the Treatment of Cervical Spinal Cord Injury
University of California, Irvine
P.I.: Hans S. Keirstead, Ph.D.
$2,400,000 for 4 years

DEAFNESS

LEON J. THAL SEED GRANT PROGRAM

Hair Cells and Spiral Ganglion Neuron Differentiation from Human Embryonic Stem Cells
University of California, Davis
P.I.: Ebenezer N. Yamoah, Ph.D.
$470,000 for 2 years

Dollar amount shown is the amount requested rounded to the nearest $5,000, not the amount awarded.

COMPREHENSIVE RESEARCH GRANT PROGRAM

Generation of inner ear sensory cells from human ES cells toward a cure for deafness
Stanford University
P.I.: Stefan Heller, Ph.D.
$2,470,000 for 4 years

EPILEPSY

COMPREHENSIVE RESEARCH GRANT PROGRAM

Derivation of Inhibitory Nerve Cells from Human Embryonic Stem Cells
University of California, San Francisco
P.I.: Arnold R. Kriegstein, M.D., Ph.D.
$2,510,000 for 4 years
Also referenced in: Parkinson's Disease

EYE DISEASE

LEON J. THAL SEED GRANT PROGRAM

Therapeutic potential of Retinal Pigment Epithelial cell lines derived from hES cells for retinal degeneration.
University of Southern California
P.I.: David R. Hinton, M.D.
$685,000 for 2 years

MULTIPLE SCLEROSIS

LEON J. THAL SEED GRANT PROGRAM

Human Embryonic Stem Cells and Remyelination in a Viral Model of Demyelination
University of California, Irvine
P.I.: Thomas Edward Lane, Ph.D.
$430,000 for 2 years

Dollar amount shown is the amount requested rounded to the nearest $5,000, not the amount awarded.

COMPREHENSIVE RESEARCH GRANT PROGRAM

Human stem cell derived oligodendrocytes for treatment of stroke and MS
University of California, San Francisco
P.I.: Samuel J. Pleasure, M.D., Ph.D.
$2,570,000 for 4 years
Also referenced in: Stroke

PARKINSON'S DISEASE

LEON J. THAL SEED GRANT PROGRAM

Identifying small molecules that stimulate the differentiation of hESCs into dopamine-producing neurons
University of California, San Francisco
P.I.: Su Guo, Ph.D.
$565,000 for 2 years

Optimization of guidance response in human embryonic stem cell derived midbrain dopaminergic neurons in development and disease
Stanford University
P.I.: Susan K. McConnell, Ph.D.
$635,000 for 2 years

Modeling Parkinson's Disease Using Human Embryonic Stem Cells
Burnham Institute for Medical Research
P.I.: Zhuohua Zhang, Ph.D.
$760,000 for 2 years

COMPREHENSIVE RESEARCH GRANT PROGRAM

Derivation of Inhibitory Nerve Cells from Human Embryonic Stem Cells
University of California, San Francisco
P.I.: Arnold R. Kriegstein, M.D., Ph.D.
$2,510,000 for 4 years
Also referenced in: Epilepsy

MEF2C-Directed Neurogenesis From Human Embryonic Stem Cells
Burnham Institute for Medical Research
P.I.: Stuart A. Lipton, M.D., Ph.D.
$3,040,000 for 4 years

Dollar amount shown is the amount requested rounded to the nearest $5,000, not the amount awarded.

ABSTRACTS

[Provided by applicant]

ALZHEIMER'S DISEASE

Generation of forebrain neurons from human embryonic stem cells

University of California, San Diego
Principal Investigator: Anirvan Ghosh, Ph.D. Leon J. Thal SEED Grant Program

The goal of this proposal is to generate forebrain neurons from human embryonic stem cells. Our general strategy is to sequentially expose ES cells to signals that lead to differentiation along a neuronal lineage, and to select for cells that display characteristics of forebrain neurons. These cells would then be used in transplantation experiments to determine if they are able to make synaptic connections with host neurons. If successful these experiments would provide a therapeutic strategy for the treatment of Alzheimer's disease and other disorders that are characterized by loss of forebrain neurons. Currently there is no effective treatments for Alzheimer's disease, and with an aging baby-boomer population, the incidence of this disease is likely to increase sharply. One of the few promising avenues to treat Alzheimer's is the possibility of cell replacement therapy in which the neurons lost could be replaced by transplanted neurons. Embryonic stem cells, which have the ability to differentiate into various cells of the body, could be a key component of such a therapy if we can successfully differentiate them into forebrain neurons.

Development of human ES cell lines as a model system for Alzheimer disease drug discovery

University of California, Irvine
Principal Investigator: Frank M. LaFerla, Ph.D. Leon J. Thal SEED Grant Program

Alzheimer disease (AD) is a progressive neurodegenerative disorder that currently affects over 4.5 million Americans. By the middle of the century, the prevalence of AD in the USA is projected to almost quadruple. As current therapies do not abate the underlying disease process, it is very likely that AD will continue to be a clinical, social, and economic burden. Progress has been made in our understanding of AD pathogenesis by studying transgenic mouse models of the disease and by utilizing primary neuronal cell cultures derived from rodents. However, key proteins that are critical to the pathogenesis of this disease exhibit many species-specific differences at both a biophysical and functional level. Additional species differences in other as yet unidentified AD-related proteins are likely to also exist.

Thus, there is an urgent need to develop novel models of AD that recapitulate the complex array of human proteins involved in this disease. Cell culture-based models that allow for rapid high-throughput screening and the identification of novel compounds and drug targets are also critically needed.

To that end we propose to model both sporadic and familial forms of AD by generating two novel human embryonic stem cell lines (hES cells). Differentiation of these lines along a neuronal lineage will provide researchers with an easily accessible and reproducible neuronal cell culture model of AD. These cells will also allow high-throughput screening and experimentation in neuronal cells with a species-relevant complement of human proteins.

In Aim 1 we will develop and characterize hES cell lines designed to model both sporadic and familial forms of AD. To model sporadic AD we will stably transfect HUES7 hES cells (developed by Douglas Melton) with lentiviral constructs coding for human wild type amyloid precursor protein (APP-695) under control of the human APP promoter. APP is well expressed within hES cells and upregulated upon

neuronal differentiation. To model familial AD and generate cells that exhibit a more aggressive formation of oligomeric A• species we will also develop a second hES cell line stably transfected with human APP that includes the Arctic (E693G) mutation.

In Aim 2 we will utilize our wild-type APP hES cells to perform a high-throughput siRNA screen. We will utilize AMAXA reverse-nucleofection in conjunction with a human druggable genome siRNA array (Dharmacon) that targets 7309 genes considered to be potential therapeutic targets. Following transfection conditioned media will be examined by a sensitive ELISA to identify novel targets that modulate A• levels. In addition a Thioflavin S assay will determine any effects on A• aggregation. Follow-up experiments will confirm promising candidates identified in the high-throughput screen. Taken together these studies aim to establish novel AD-specific hES cell lines and identify promising new therapeutic targets for this devastating disease.

Using Human Embryonic Stem Cells to Understand and to Develop New Therapies for Alzheimer's Disease

University of California, San Diego
Principal Investigator: Lawrence S. B. Goldstein, Ph.D. Comprehensive Research Program

Alzheimer's Disease (AD) is a progressive incurable disease that robs people of their memory and ability to think and reason. It is emotionally, and sometimes financially devastating to families that must cope when a parent or spouse develops AD. Unfortunately, however, we currently lack an understanding of Alzheimer's Disease (AD) that is sufficient to drive the development of a broad range of therapeutic strategies. Compared to diseases such as cancer or heart disease, which are treated with a variety of therapies, AD lacks even one major effective therapeutic approach. A key problem is that there is a paucity of predictive therapeutic hypotheses driving the development of new therapies. Thus, there is tremendous need to better understand the cellular basis of AD so that effective drug and other therapies can be developed. Several key clues come from rare familial forms of AD (FAD), which identify genes that can cause disease when mutant and which have led to the leading hypotheses for AD development. Recent work on Drosophila and mouse models of Alzheimer's Disease (AD) has led to a new suggestion that early defects in the physical transport system that is responsible for long-distance movements of vital supplies and information in neurons causes neuronal dysfunction. The type of neuronal failure caused by failures of the transport systems is predicted to initiate an autocatalytic spiral of biochemical events terminating in the classic pathologies, i.e., plaques and tangles, and the cognitive losses characteristic of AD. The problem, however, is how to test this new model and the prevailing "amyloid cascade" model, or indeed any model of human disease developed from studies in animal models, in humans. It is well known that mouse models of AD do not fully recapitulate the human disease, perhaps in part because of human-specific differences that alter the details of the biochemistry and cell biology of human neurons. One powerful approach to this problem is to use human embryonic stem cells to generate human neuronal models of hereditary AD to test rigorously the various hypotheses. These cellular models will also become crucial reagents for finding and testing new drugs for the treatment of AD.

AMYOTROPHIC LATERAL SCLEROSIS (ALS) /SPINAL MUSCULAR ATROPHY (SMA)

In vitro differentiation of hESCs into corticospinal motor neurons

University of California, Santa Cruz
Principal Investigator: Bin Chen, Ph.D. Leon J. Thal SEED Grant Program

Amyotrophic lateral sclerosis (ALS) is a rapidly progressive, fatal neurological disease that leads to the degeneration of motor neurons in the brain and in the spinal cord. There are currently 20,000 ALS patients in the United States, and 5,000 new patients are diagnosed every year. Unfortunately no cure has been found for ALS. The only medication approved by the FDA to treat ALS can only slow the disease's progression and prolong life by a few months in some patients. Thus it is critical to explore other therapeutic strategies for the treatment of ALS such as cell replacement strategy.

Because of the ability to generate many different cell types, human embryonic stem cells (hESCs) may potentially serve as a renewable source of cells for replacing the damaged cells in diseases. However, transplanting ESCs directly may cause tumor growth in patients. To support cell transplants, it is important to develop methods to differentiate hESCs into the specific cell types affected by the disease. In this application, we propose to develop an effective method to differentiate hESCs into corticospinal motor neurons (CSMNs), the neurons in the cerebral cortex that degenerate in ALS. We will test whether these CSMNs generated from hESCs in culture conditions can form proper connections to the spinal cord when transplanted into mouse brains.

To direct hESCs to become the CSMNs, it is critical to establish a reliable method to identify human CSMNs. Recent progress in developmental neuroscience have identified genes that are specifically expressed in the CSMNs in mice. However no information is available for identifying human CSMNs. We hypothesize that CSMN genes in mice will be reliable markers for human CSMNs. To test this hypothesis we will investigate whether mouse CSMN markers are specifically expressed in the human CSMNs.

The therapeutic application of hESCs to replace damaged CSMNs in ALS depends on the ability to direct hESCs to develop into CSMNs. Currently a reliable condition to direct hESCs to differentiate into CSMNs has not been established. We will attempt to differentiate hESCs into CSMNs based on the knowledge gained from studying the development of nervous system. We will achieve this goal in two steps: first we will culture hESCs in a condition to make them become progenitors cells of the most anterior region of the brain; then we will culture these progenitors to become neurons of the cerebral cortex, particularly the CSMNs. We will study the identities of these neurons using the CSMN markers that we have proposed to identify.

To apply the cell replacement strategy to treat ALS, it will be critical to test if human CSMNs generated from cultured hESCs can form proper connections in an animal model. We will transplant the CSMNs developed from hESCs into the brains of mice and test whether they can form connections to the spinal cord.

When carried out, the proposed research will directly benefit cell replacement therapy for ALS.

Retinoic Acid-FGF Antagonism during Motor Neuron Differentiation of Human ES Cells
Burnham Institute for Medical Research
Principal Investigator: Gregg Duester, Ph.D. Leon J. Thal SEED Grant Program

Retinoic acid is a metabolic derivative of vitamin A that has recently been shown to stimulate differentiation of human embryonic stem cells into motor neurons. However, almost nothing is known about how retinoic acid may perform this function. The recent discovery that retinoic acid antagonizes the action of fibroblast growth factor suggests a possible mechanism for retinoic acid function during motor neuron differentiation. We plan to use our knowledge of retinoic acid-fibroblast growth factor interaction to understand how retinoic acid stimulates human embryonic stem cells to go down the motor neuron lineage. Such knowledge will allow us to devise rational strategies for optimal use of retinoic with other reagents to reliably differentiate human embryonic stem cells into motor neurons. Our studies will contribute to the development of cell-replacement therapies for motor neuron loss in patients with amyotrophic lateral sclerosis or spinal cord injury. We plan to study the effect of retinoic acid on differentiation of human embryonic stem cell lines that are ineligible for federal funding. Because all of the human embryonic stem cell lines approved for federal funding were generated using methods that involved mixing human cells with mouse feeder cells, they are poorly suited for clinical use and are likely to have undergone modifications with unpredictable consequences. Given the scarcity of federally-approved human embryonic stem cell lines, and recognizing that cells passaged for long periods of time in culture tend to become genetically unstable, it is necessary to have the means of characterizing new human embryonic stem cell lines. The studies proposed here on human embryonic stem cell lines that are ineligible for federal funding will provide new insight into how retinoic acid and fibroblast growth factor can best be used to generate motor neurons for therapeutic purposes.

Gene regulatory mechanisms that control spinal neuron differentiation from hES cells.
The Salk Institute for Biological Studies
Principal Investigator: Samuel L. Pfaff, Ph.D. Leon J. Thal SEED Grant Program

More than 600 disorders afflict the nervous system. Common disorders such as stroke, epilepsy, Parkinson's disease and autism are well-known. Many other neurological disorders are rare, known only to the patients and families affected, their doctors and scientists who look to rare disorders for clues to a general understanding of the brain as well as for treatments for specific diseases. Neurological disorders strike an estimated 50 million Americans each year, exacting an incalculable personal toll and an annual economic cost of hundreds of billions of dollars in medical expenses and lost productivity. There are many potential applications for using human embryonic stem (hES) cells to treat neurological diseases and injuries; however, a critical barrier to progress in the field is the ability to efficiently and reliably control neuronal differentiation from these cells. The main goal of this proposal is to define the gene regulatory mechanisms that control the acquisition of neuronal fate from hES cells. Longer term, we plan to produce small compounds (drugs) that greatly facilitate this process. Drugs that enhance neuron formation are likely to improve scientists' ability to manipulate hES cells and create in vitro models for studying neurological diseases. Most importantly, drugs of this type may stimulate endogenous stem cells within adults to self-repair damaged areas of the brain.

Because so little is known about how hES cells differentiate into neurons at the molecular level, this grant will focus on understanding how a single neuronal subtype is generated - motor neurons. Why motor neurons? Motor neuron diseases are a group of progressive neurological disorders that destroy cells that control essential muscle activity such as speaking, walking, breathing and swallowing. Eventually, the ability to control voluntary movement can be lost. Motor neuron diseases may be inherited or acquired, and they occur in all age groups. In adults, symptoms often appear after age 40. In children, particularly

in inherited or familial forms of the disease, symptoms can be present at birth or appear before the child learns to walk. Is there a treatment? There is no cure or standard treatment for motor neuron diseases. Prognosis varies depending on the type of motor neuron disease and the age of onset; however, many types such as ALS and some forms of spinal muscular atrophy are typically fatal.

The experiments in this proposal seek to understand mechanisms that will be directly applicable to hES cells and their use for treating motor neuron diseases. Moreover, the mechanisms controlly motor neuron formation are also likely to be relevant to many other neuronal subtypes. Therefore, these studies should provide essential and general insight into medically deploying strategies for converting hES cells into specific neuronal subtypes and thereby serve as a platform for treating a wide range of neurological diseases.

Genetic manipulation of human embryonic stem cells and its application in studying CNS development and repair

University of California, San Diego

Principal Investigator: Binhai Zheng, Ph.D. Leon J. Thal SEED Grant Program

Also referenced in: Spinal Cord Injury

The advent of human embryonic stem cells (hESCs) has offered enormous potential for regenerative medicine and for basic understanding of human biology. On the one hand, hESCs can be turned into many different cell types in culture dish, and specific cell types derived from hESCs offer an almost infinite source for cellular replacement therapies. This is the primary reason for which hESCs have received much attention from the general public. On the other hand, scientists can study the properties of hESCs and their derivatives, and determine the effect of genes and molecules on such properties either in culture dish or with transplantation studies in live animals. This second aspect of hESC research would not only significantly enhance our understanding of the function of human genes, but will greatly augment our ability to apply hESCs in transplantation therapies and regenerative medicine. To attain the full potential of hESCs, genetic manipulation of hESCs is essential. In this proposal, we will establish the methods to genetically manipulate an increasingly used, non-federally approved hESC line, the HUES-9, and assess the feasibility to use genetically modified HUES-9 cells in cell transplantation studies to assess the integration of hESCs into the mouse central nervous system. We propose to achieve both homologous recombination (i.e. gene targeting) and transgene expression (with bacterial artificial chromosome), which have complementary utilities in assaying gene function in addition to the opportunity to label hESCs or their derivatives with fluorescent markers. Specifically, with genetic engineering of hESCs we will be able to 1) label hESCs and specific cell types derived from hESCs so that they can be readily followed in culture dish and in animals that have received cellular transplants; 2) disturb an endogenous gene or add more copies of a gene so that the effect of a gene of interest can be assessed (for this purpose, a gene involved in the development of a major motor tract, the corticospinal tract, will be studied). We will then transplant genetically engineered hESCs and their derivatives into the embryonic and adult mouse CNS to assess how well these cells integrate into the mouse CNS, and whether such transplanted animals can serve as valid models to study the effect of genes on hESC function in live animals. In transplantation studies involving adult mouse recipients, injured mouse CNS will be used in addition to intact CNS in order to evaluate the potential of hESCs to integrate into injured CNS, which has direct implications on the therapeutic potential of these cells. In summary, our proposal will establish the methods and tools to genetically manipulate HUES-9 cells, explore a paradigm to study human genes and cells in a context of neural development and cellular therapies, and will pave the way for future studies of genes and pathways in basic biology and regenerative medicine with hESCs.

Molecular and Cellular Transitions from ES Cells to Mature Functioning Human Neurons
The Salk Institute for Biological Studies
Principal Investigator: Fred H. Gage, Ph.D. Comprehensive Research Grant Program

Human embryonic stem cells (hESCs) are pluripotent entities, capable of generating a whole-body spectrum of distinct cell types. We have developmental procedures for inducing hESCs to develop into pure populations of human neural stem cells (hNS), a step required for generating authentic mature human neurons. Several protocols have currently been developed to differentiate hESCs to what appear to be differentiated dopaminergic neurons (important in Parkinson's disease (PD) and cholinergic motor neurons (important in Amyolateral Sclerosis (ALS) in culture dishes. We have developed methods to stably insert new genes in hESC and we have demonstrated that these transgenic cells can become mature neurons in culture dishes. We plan to over express alpha synuclein and other genes associated with PD and superoxide dismutase (a gene mutated in ALS) into hESCs and then differentiate these cells to neurons, and more specifically to dopaminergic neurons and cholinergic neurons using existing protocols. These transgenic cells can be used not only for the discovery of cellular and molecular causes for dopaminergic or cholinergic cell damage and death in these devastating diseases, but also can be used as assays to screen chemical libraries to find novels drugs that may protect against the degenerative process. Until recently the investigation of the differentiation of these human cells has only been observed in culture dishes or during tumor formation. Our recent results show that hESC implanted in the brains of mice can survive and become active functional human neurons that successfully integrate into the adult mouse forebrain. This method of transplantation to generate models of human disease will permit the study of human neural development in a living environment, paving the way for the generation of new models of human neurodegenerative and psychiatric diseases. It also has the potential to speed up the screening process for therapeutic drugs.

hESC-Derived Motor Neurons For the Treatment of Cervical Spinal Cord Injury
University of California, Irvine
Principal Investigator: Hans S. Keirstead, Ph.D. Comprehensive Research Grant Program
Also referenced in: Spinal Cord Injury

Cervical spinal cord injuries result in a loss of upper limb function because the cells within the spinal cord that control upper limb muscles are destroyed. The goal of this research program is to create a renewable human source of these cells, to restore upper limb function in both acute and chronic spinal cord injuries. There are two primary challenges to the realization of this goal: 1) a source of these human cells in high purity, and 2) functional integration of these cells in the body after transplantation.

Human embryonic stem cells (hESCs) can form any cell in the body, and can reproduce themselves almost indefinitely to generate large quantities of human tissue. One of the greatest challenges of hESC research is to find ways to restrict hESCs such that they generate large amounts on only one cell type in high purity such that they could be used to replace lost cells in disease or trauma. Our laboratory was the first laboratory in the world to develop a method to restrict hESCs such that they generate large amounts of only one cell type in high purity. That cell type is called an oligodendrocyte, which insulates connections in the spinal cord to allow them to conduct electricity. Transplantation of these cells was useful for treating spinal cord injuries in rats if the treatment was given one week after the injury. That treatment is being developed for use in humans.

Recent studies in our laboratory indicate that we have succeeded in restricting hESCs to generate large quantities of a different cell type in the spinal cord, that which controls upper limb muscles. We have generated large quantities of these human cells, grown them with human muscle, and demonstrated that they connect and control the human muscle. The cells also express markers that are appropriate for this cell type.

Here we propose to generate these cells in high purity from hESCs and genetically modify them so that they can be induced to grow over inhibitory environments that exist in the injured spinal cord. We will then determine whether these human cells have the ability to regenerate the injured tissue in the spinal cord, and restore lost function. All of our studies will be conducted in an FDA-compliant manner, which will speed the translation of our results to humans if we are successful. The studies outlined in this proposal represent a novel approach to treating spinal cord injury, which might work for both acute and chronic injuries.

DEAFNESS

Hair Cells and Spiral Ganglion Neuron Differentiation from Human Embryonic Stem Cells

University of California, Davis
Principal Investigator: Ebenezer N. Yamoah, Ph.D. Leon J. Thal SEED Grant Program

Hair cells (HCs) convert sound and balance signals into electrical impulses in the inner ear, including the cochlea and the vestibular endorgans, with remarkable precision and sensitivity. Our long-term goal is to stimulate HC regeneration in human inner ears and to enable the functional innervations of HCs by neurons. Hair cells are terminally-differentiated cells. Once HCs are lost due to noise, ototoxic drugs or aging, there is no effective way to stimulate HC regeneration in mature inner ears. However, recent studies from our group and others have demonstrated very encouraging results: new HCs may be formed from stem cells.

We know very little about how to induce HC regeneration in a mature sensory epithelia in the auditory and vestibular organs. Indeed, determination of the mechanisms of induction of HCs and the assembly of the functional machinery of HCs in the mature cochlea has direct relevance to our understanding of how a HC may be derived from specific human embryonic stem cells (hESCs). Strong evidence from data in developmental cell biology and electrophysiology motivates our hypothesis that the specific factors regulating HC differentiation interact to confer their functions and that specific hESC-types have the potential to differentiate into HCs and their innervating neurons. We further predict that newly differentiated HCs assemble their transduction apparatus and ionic currents in a coordinated fashion to achieve the cell's sensitivity.

The proposed research will identify some of the candidate proteins and their mechanisms of interactions that are required to induce HC differentiation from hESCs. The project will determine the hESC-types, which have the competence to transform into HCs. We will assess whether a HC assembles its entire transduction apparatus and ionic conductance simultaneously, at a specific stage in the process of differentiation, or whether the assembly of the final apparatus entails multiple steps during maturation. Moreover, these studies should reveal how HCs and neurons coordinate and regulate the mechano-electrical apparatus, information that might be exploited to induce regeneration and functional transduction apparatus assembly from hESCs after HC damage.

Of particular importance to auditory and vestibular science is the possibility that a rational design of a cocktail of protein/factors may be assembled for 'biological implants', as our understanding of the mechanisms of regeneration of HCs becomes more refined. Since the mechanisms used by the internal ear and hESCs may be expressed in different forms by other signal transduction systems, these studies may provide novel insights into such areas as protein-protein interaction, cell proliferation, developmental processes, and hESC signaling in general.

Generation of inner ear sensory cells from human ES cells toward a cure for deafness

Stanford University
Principal Investigator: Stefan Heller, Ph.D. Comprehensive Research Grant Program

Hearing loss is the leading birth defect in the United States with ~3 children in 1,000 born with partial to profound compromise of auditory function. Debilitating hearing loss is estimated to affect ~4% of people under 45 years of age, and 34% of those 65 years or over.

A major cause of why acquired hearing loss is permanent in mammals lies in the incapacity of the sensory epithelia of the inner ear to replace damaged mechanoreceptor cells, or hair cells. Sensory hair

cells are mechanoreceptors that transduce fluid movements generated by sound into electrochemical signals interpretable by the brain. Degeneration and death of hair cells is causal in >80% of individuals with hearing loss

In this grant application, we propose to explore, in comparative manner, the potential of at least five human ESC lines to develop into hair cells. We strive to use recently derived human embryonic stem cells for this purpose to avoid problems caused by potential chromosomal abnormalities and nonhuman or viral contaminants, which greatly restrict the use of these stem cells and render their derivatives unacceptable for in vivo studies. Federal funding cannot be used for research with these embryonic stem cell lines.

The most exciting long-term goal of the proposed experimentation is to provide an abundant source of human inner ear progenitor cells that can be tapped in the future to routinely create human hair cells for in vitro and in vivo experiments and for clinical studies aimed to repair damaged ears. Having access to human hair cells in vitro offers, for the first time, the opportunity for detailed cell-biological studies of this cell type. We envision that human ESC-derived inner ear progenitor cells can be used to screen for drugs that lead to increased hair cell differentiation. Equally exiting with regard to possible clinical applications are studies aimed at differentiating functional human hair cells in vitro, in organ culture, and in vivo after transplantation of the cells into the cochleae of deaf animal models and potentially into human patients. In the more distant future, we envisage that our proposed research will result in novel treatment strategies to cure deafness and potentially other inner ear diseases such as tinnitus caused by malfunctioning sensory hair cells, and vestibular disorders.

EPILEPSY

Derivation of Inhibitory Nerve Cells from Human Embryonic Stem Cells

University of California, San Francisco
Principal Investigator: Arnold R. Kriegstein, M.D., Ph.D. Comprehensive Research Program
Also referenced in: Parkinson's Disease

Parkinson's disease (PD) is caused by degeneration of a specific population of dopamine-producing nerve cells in the brain and is chronic, progressive, and incurable. Loss of dopamine-containing cells results in profound physiological disturbances producing tremors, rigidity, and severe deterioration of gate and balance. In the United States, approximately 1.5 million people suffer with PD and it is estimated that 60,000 new cases are diagnosed each year. Drugs can modify some of the disease symptoms, but many patients develop disabling drug-induced movements that are unresponsive to medication. Deep brain stimulation can alleviate motor symptoms in some patients but is not a cure. We plan an entirely novel approach to treat PD. We propose to utilize a specific class of inhibitory nerve cells found in the embryonic brain, known as MGE cells, as donor transplant cells to inhibit those brain regions whose activity is abnormally increased in PD. In preliminary studies we have demonstrated that this approach can relieve symptoms in an animal model of PD. To turn this approach into a patient therapy, we will need to develop methods to obtain large numbers of human cells suitable for transplantation. This proposal seeks to address this problem by producing unlimited numbers of exactly the right type of MGE nerve cell using human embryonic stem cells.

The inhibitory nerve cells we seek to produce will reduce brain activity in target regions. They may therefore be used to treat other conditions characterized by excessive brain activity, such as epilepsy. Epilepsy can be a life threatening and disabling condition. Nearly two million Americans suffer with some form of epilepsy. Unfortunately, modulation of brain excitability using antiepileptic drugs can have serious side-effects, especially in the developing brain, and many patients can only be improved by surgically removing areas of the brain containing the seizure focus. Using MGE cells made from human embryonic stem cell lines, we hope to develop a novel epilepsy treatment that could replace the need for surgery or possibly even drug therapy.

We propose an integrated approach that combines the complementary expertise of four UCSF laboratories to achieve our goals. We have already determined that mouse MGE cells can improve the symptoms of PD and epilepsy when grafted into animal models. We now need to develop methods to obtain large numbers of human cells suitable for grafting. We need to ensure that when delivered, the cells will migrate and integrate in the target brain regions, and we need to evaluate therapeutic efficacy in animal models of Parkinson's disease and epilepsy. This proposal addresses these goals. If successful, this accomplishment will set the stage for studies in primates and hasten the day when MGE cells may be used as patient therapy for a wide variety of debilitating neurological disorders.

EYE DISEASE

Therapeutic potential of Retinal Pigment Epithelial cell lines derived from hES cells for retinal degeneration.

University of Southern California
Principal Investigator: David R. Hinton, M.D. Leon J. Thal SEED Grant Program

Retinal degeneration represents a group of blinding diseases that are increasingly impacting the health and well being of Californians. It is estimated that by 2020, over 450,000 Californians will suffer from vision loss or blindness due to the age-related macular degeneration (AMD), the most common cause of retinal degeneration diseases in the elderly. Furthermore, retinitis pigmentosa is the leading cause of inherited blindness in younger people. Currently there are no cures for these diseases.

A layer of cells at the back of the eye called the retinal pigment epithelium (RPE), provide support, protection, and nutrition to the light sensitive retina, and cooperate with other retinal cells to maintain normal visual function. The dysfunction and/or loss of these RPE cells play a critical role in the development of the previously described blinding diseases. We suggest that effective treatment of retinal degeneration could be achieved by the proper replacement of damaged RPE and retinal cells with healthy ones. However, lack of the reasonable supply of healthy human eye cells hampers the application of this therapeutic approach.

Recent advances in knowledge and technology of embryonic stem cells brings new hope for the development of cell replacement treatment. Embryonic stem (ES) cells are capable of unlimited self-replication and production of different cell types. RPE cells derived from human ES cells (hES-RPE) are a potentially unlimited resource for the cell replacement approach.

We hypothesize that the dysfunction and/or loss of RPE can be replenished and restored through the transplantation of functionally polarized RPE monolayers derived from human embryonic stem cells, and this transplantation can cure the retinal degeneration diseases caused by RPE dysfunction.

We propose to: 1. Derive RPE cells from human ES cells; 2. Establish and characterize the functionally mature or polarized monolayer of hES-RPE cells that will be suitable for transplantation; 3. Rescue the retinal degeneration phenotype through the transplantation of functionally mature or polarized monolayer of hES-RPE cells in animal models. Our goal is to determine the feasibility of treating the retinal degeneration diseases caused by RPE dysfunction through the transplantation of a monolayer of polarized hES-RPE cell sheet.

The knowledge and technology from our research can be used to develop new treatments for human retinal degeneration diseases.

MULTIPLE SCLEROSIS

Human Embryonic Stem Cells and Remyelination in a Viral Model of Demyelination
University of California, Irvine
Principal Investigator: Thomas Edward Lane, Ph.D. Leon J. Thal SEED Grant Program

Multiple sclerosis (MS) is the most common neurologic disease affecting young adults under the age of 40 with the majority of MS patients diagnosed in the second or third decade of life. MS is characterized by the gradual loss of the myelin sheath that surrounds and insulates axons that allow for the conduction of nerve impulses- a process known as demyelination. For unknown reasons, the ability to remyelinate axons is impaired in MS patients making recovery of motor skills difficult. Therefore, developing novel and effective approaches to remyelinate axons in MS patients would dramatically improve the quality of life of many MS patients. The experiments described in this research proposal utilize a well-accepted model of MS to further characterize the potential clinical applicability of human embryonic stem cells (hESCs) to remyelinate axons. Such knowledge is crucial in order to increase our understanding of stem cells with regards to treatment of numerous human diseases including MS.

Human stem cell derived oligodendrocytes for treatment of stroke and MS
University of California, San Francisco
Principal Investigator: Samuel J. Pleasure, M.D., Ph.D. Comprehensive Research Program
Also referenced in: Stroke

Strokes that affect the nerves cells, i.e., 'gray matter', consistently receive the most attention. However, the kind of strokes that affecting the 'wiring' of the brain, i.e., 'white matter', cause nearly as much disability. The most severe disability is caused when the stroke is in the wiring (axons) that connect the brain and spinal cord; as many as 150,000 patients are disabled per year in the US from this type of stroke. Although oligodendrocytes ('oligos' are the white matter cells that produce the lipid rich axonal insulator called myelin) are preferentially damaged during these events, stem cell-derived oligos have not been tested for their efficacy in preclinical (animal) trials. These same white matter tracts (located underneath the gray matter, called subcortical) are also the primary sites of injury in MS, where multifocal inflammatory attack is responsible for stripping the insulating myelin sheaths from axons resulting in axonal dysfunction and degeneration. Attempts to treat MS-like lesions in animals using undifferentiated stem cell transplants are promising, but most evidence suggests that these approaches work by changing the inflammation response (immunomodulation) rather than myelin regeneration. While immunomodulation is unlikely to be sufficient to treat the disease completely, MS may not be amenable to localized oligo transplantation since it is such a multifocal process. This has led to new emphasis on approaches designed to maximize the response of endogenous oligo precursors that may be able to regenerate myelin if stimulated. We hypothesize that by exploiting novel features of oligo differentiation in vitro (that we have discovered and that are described in our preliminary data) that we will be able to improve our ability to generate oligo lineage cells from human embryonic stem cells and neural stem cells for transplantation, and also to develop approaches to maximize oligo development from endogenous precursors at the site of injury in the brain. This proposal will build on our recent successes in driving oligo precursor production from multipotential mouse neural stem cells by expressing regulatory transcription factors, and apply this approach to human embryonic and neural stem cells to produce cells that will be tested for their ability to ameliorate brain damage in rodent models of human stroke. Furthermore, we hope to develop approaches that may facilitate endogenous recruitment of oligo precursors to produce mature oligos, which may prove a viable regenerative approach to treat a variety of white matter diseases including MS and stroke.

PARKINSON'S DISEASE

Identifying small molecules that stimulate the differentiation of hESCs into dopamine-producing neurons

University of California, San Francisco
Principal Investigator: Su Guo, Ph.D. Leon J. Thal SEED Grant Program

In this application, we propose to identify small molecule compounds that can stimulate human embryonic stem cells to become dopamine-producing neurons. These neurons degenerate in Parkinson's disease, and currently have very limited availability, thus hindering the cell replacement therapy for treating Parkinson's disease.

Our proposed research, if successful, will lead to the identification of small molecule compounds that can not only stimulate cultured human embryonic stem cells to become DA neurons, but may also stimulate endogenous brain stem cells to regenerate, since the small molecule compounds can be made readily available to the brain due to their ability to cross the blood-brain barrier. In addition, these small molecule compounds may serve as important research tools, which can tell us the fundamental biology of the human embryonic stem cells.

Optimization of guidance response in human embryonic stem cell derived midbrain dopaminergic neurons in development and disease

Stanford University
Principal Investigator: Susan K. McConnell, Ph.D. Leon J. Thal SEED Grant Program

A promising approach to alleviating the symptoms of Parkinson's disease is to transplant healthy dopaminergic neurons into the brains of these patients. Due to the large number of transplant neurons required for each patient and the difficulty in obtaining these neurons from human tissue, the most viable TRANSPLANTATION STRATEGY WILL UTILIZE NOT FETAL DOPAMINERGIC NEURONS BUT DOPAMINERGIC NEURONS DERIVED FROM human stem cell lines. While transplantation has been promising, it has had limited success, in part due to the ability of the new neurons to find their correct targets in the brain. This incorrect targeting may be due to the lack of appropriate growth and guidance cues as well as to inflammation in the brain that occurs in response to transplantation, or to a combination of the two. Cytokines released upon inflammation can affect the ability of the new neurons to connect, and thus ultimately will affect their biological function. In out laboratory we have had ongoing efforts to determine the which guidance molecules are required for proper targeting of dopaminergic neurons during normal development and we have identified necessary cues. We now plan to extend these studies to determine how these critical guidance cues affect human stem cell derived dopaminergic neurons, the cells that will be used in transplantation. In addition, we will examine how these guidance cues affect both normal and stem cell derived dopaminergic neurons under conditions that are similar to the diseased and transplanted brain, specifically when the brain is inflamed. Ultimately, an understanding of how the environment of the transplanted brain influences the ability of the healthy new neurons to connect to their correct targets will lead to genetic, and/or drug-based strategies for optimizing transplantation therapy.

Modeling Parkinson's Disease Using Human Embryonic Stem Cells

Burnham Institute for Medical Research
Principal Investigator: Zhuohua Zhang, Ph.D. Leon J. Thal SEED Grant Program

Parkinson's disease (PD) is the most frequent neurodegenerative movement disorder caused by damage of dopamine-producing nerve cells (DA neuron) in patient brain. The main symptoms of PD are age-

dependent tremors (shakiness). There is no cure for PD despite administration of levodopa can help to control symptoms.

Most of PD cases are sporadic in the general population. However, about 10-15% of PD cases show familial history. Genetic studies of familial cases resulted in identification of PD-linked gene changes, namely mutations, in six different genes, including α-synuclein, LRRK2, uchL1, parkin, PINK1, and DJ-1. Nevertheless, it is not known how abnormality in these genes cause PD. Our long-term research goal is to understand PD pathogenesis at cellular and molecular levels via studying functions of these PD-linked genes and dysfunction of their disease-associated genetic variants.

A proper experimental model plays critical roles in defining pathogenic mechanisms of diseases and for developing therapy. A number of cellular and animal models have been developed for PD research. Nevertheless, a model closely resembling generation processes of human DA nerve cells is not available because human neurons are unable to continuously propagate in culture. Nevertheless, human embryonic stem cells (hESCs) provide an opportunity to fulfill the task. hESCs can grow and be programmed to generate DA nerve cells. In this study, we propose to create a PD model using hESCs. The strategy is to express PD pathogenic mutants of α-synuclein or LRRK2 genes in hESCs. Mutations in α-synuclein or LRRK2 genes cause both familial and sporadic PD. α-Synuclein is a major component of Lewy body, aggregates found in the PD brain. The model will allow us to determine molecular action of PD pathogenic α-synuclein and LRRK2 mutants during generation of human DA neuron and interactions of PD related genes and environmental toxins in DA neurons derived from hESCs.

Our working hypothesis is that PD associated genes function in hESCs-derived DA neurons as in human brain DA neurons. Pathogenic mutations in combination with environmental factors (i.e. aging and oxidative stress) impair hESCs-derived DA function resulting in eventual selective neuronal death. In this study, we will firstly generate PD cellular models via expressing two PD-pathogenic genes, α-synuclein and LRRK2 in hESCs. We will next determine effects of α-synuclein and LRRK2 on hESCs and neurons derived from these cells. Finally, we will determine whether PD-causing toxins (i.e. MPP+, paraquat, and rotenone) selectively target to DA neurons derived from hESCs. Successful completion of this study will allow us to study the pathological mechanism of PD and to design strategies to treat the disease.

Derivation of Inhibitory Nerve Cells from Human Embryonic Stem Cells

University of California, San Francisco
Principle Investigator: Arnold R. Kriegstein, M.D., Ph.D. Comprehensive Research Program
Also referenced in: Epilepsy

Parkinson's disease (PD) is caused by degeneration of a specific population of dopamine-producing nerve cells in the brain and is chronic, progressive, and incurable. Loss of dopamine-containing cells results in profound physiological disturbances producing tremors, rigidity, and severe deterioration of gate and balance. In the United States, approximately 1.5 million people suffer with PD and it is estimated that 60,000 new cases are diagnosed each year. Drugs can modify some of the disease symptoms, but many patients develop disabling drug-induced movements that are unresponsive to medication. Deep brain stimulation can alleviate motor symptoms in some patients but is not a cure. We plan an entirely novel approach to treat PD. We propose to utilize a specific class of inhibitory nerve cells found in the embryonic brain, known as MGE cells, as donor transplant cells to inhibit those brain regions whose activity is abnormally increased in PD. In preliminary studies we have demonstrated that this approach can relieve symptoms in an animal model of PD. To turn this approach into a patient therapy, we will need to develop methods to obtain large numbers of human cells suitable for transplantation. This proposal seeks to address this problem by producing unlimited numbers of exactly the right type of MGE nerve cell using human embryonic stem cells.

The inhibitory nerve cells we seek to produce will reduce brain activity in target regions. They may therefore be used to treat other conditions characterized by excessive brain activity, such as epilepsy. Epilepsy can be a life threatening and disabling condition. Nearly two million Americans suffer with some form of epilepsy. Unfortunately, modulation of brain excitability using antiepileptic drugs can have serious side-effects, especially in the developing brain, and many patients can only be improved by surgically removing areas of the brain containing the seizure focus. Using MGE cells made from human embryonic stem cell lines, we hope to develop a novel epilepsy treatment that could replace the need for surgery or possibly even drug therapy.

We propose an integrated approach that combines the complementary expertise of four UCSF laboratories to achieve our goals. We have already determined that mouse MGE cells can improve the symptoms of PD and epilepsy when grafted into animal models. We now need to develop methods to obtain large numbers of human cells suitable for grafting. We need to ensure that when delivered, the cells will migrate and integrate in the target brain regions, and we need to evaluate therapeutic efficacy in animal models of Parkinson's disease and epilepsy. This proposal addresses these goals. If successful, this accomplishment will set the stage for studies in primates and hasten the day when MGE cells may be used as patient therapy for a wide variety of debilitating neurological disorders.

MEF2C-Directed Neurogenesis From Human Embryonic Stem Cells
Burnham Institute for Medical Research
Principal Investigator: Stuart A. Lipton, M.D., Ph.D. Comprehensive Research Grant Program
Also referenced in: Stroke

Understanding differentiation of human embryonic stem cells (hESCs) provides insight into early human development and will help directing hESC differentiation for future cell-based therapies of Parkinson's disease, stroke and other neurodegenerative conditions.

The PI's laboratory was the first to clone and characterize the transcription factor MEF2C, a protein that can direct the orchestra of genes to produce a particular type of cell, in this case a nerve cell (or neuron). We have demonstrated that MEF2C directs the differentiation of mouse ES cells into neurons and suppresses glial fate. MEF2C also helps keep new nerve cells alive, which is very helpful for their successful transplantation. However, little is known about the role of MEF2C in human neurogenesis, that is, its ability to direct hESC differentiation into neuronal lineages such as dopaminergic neurons to treat Parkinson's disease and its therapeutic potential to promote the generation of nerve cells in stem cell transplantation experiments. The goal of this application is to fill these gaps.

The co-PI's laboratory has recently developed a unique procedure for the efficient differentiation of hESCs into a uniform population of neural precursor cells (NPCs), which are progenitor cells that develop from embryonic stem cells and can form different kinds of mature cells in the nervous system. Here, we will investigate if MEF2C can instruct hESC-derived NPCs to differentiate into nerve cells, including dopaminergic nerve cells for Parkinson's disease or other types of neurons that are lost after a stroke. Moreover, we will transplant hESC-NPCs engineered with MEF2C to try to treat animal models of stroke and Parkinson's disease. We will characterize known and novel MEF2C target genes to identify critical components in the MEF2C transcriptional network in the clinically relevant cell population of hESC-derived neural precursor cells (hESC-NPCs).

Specifically we will: 1) determine the function of MEF2C during in vitro neurogenesis (generation of new nerve cells) from hESC-NPCs; 2) investigate the therapeutic potential of MEF2C engineered hESC-NPCs in Parkinson's and stroke models; 3) determine the MEF2C DNA (gene) binding sites and perform a "network" analysis of MEF2C target genes in order to understand how MEF2C works in driving the formation of new nerve cells from hESCs.

INJURIES TO THE NERVOUS SYSTEM

Damage to the nervous system caused by stroke, traumatic brain injury or spinal cord injury is a leading cause of disability in adults but can also afflict children. Several recent studies show that injection of stem cells or their derivatives can improve motor function in rodents following spinal cord injury. Advancing a stem cell-based treatment from animal experiments to practical therapies in humans requires that the stem cells integrate successfully into damaged tissue, withstand the challenging environment of damaged nervous tissue, and mature into functional cells of the nervous system. In 2007, 11 awards are approved for funding to address these challenges.

SPINAL CORD INJURY

LEON J. THAL SEED GRANT PROGRAM

The Immunological Niche: Effect of immunosuppressant drugs on stem cell proliferation, gene expression, and differentiation in a model of spinal cord injury.
University of California, Irvine
P.I.: Brian John Cummings, Ph.D.
$620,000 for 2 years

New Chemokine-Derived Therapeutics Targeting Stem Cell Migration
Burnham Institute for Medical Research
P.I.: Ziwei Huang, Ph.D.
$760,000 for 2 years
Also referenced in: Traumatic Brain Injury, Stroke

Genetic manipulation of human embryonic stem cells and its application in studying CNS development and repair
University of California, San Diego
P.I.: Binhai Zheng, Ph.D.
$645,000 for 2 years
Also referenced in: Amyotrophic lateral sclerosis (ALS) /Spinal Muscular Atrophy

COMPREHENSIVE RESEARCH GRANT PROGRAM

hESC-Derived Motor Neurons For the Treatment of Cervical Spinal Cord Injury
University of California, Irvine
P.I.: Hans S. Keirstead, Ph.D.
$2,400,000 for 4 years
Also referenced in: Amyotrophic lateral sclerosis (ALS) /Spinal Muscular Atrophy

Spinal ischemic paraplegia: modulation by human embryonic stem cell implant.
University of California, San Diego
P.I.: Martin Marsala, M.D.
$2,450,000 for 4 years

Dollar amount shown is the amount requested rounded to the nearest $5,000, not the amount awarded.

STROKE

LEON J. THAL SEED GRANT PROGRAM

EC regeneration in cerebrovascular ischemia: role of NO
Stanford University
P.I.: John P. Cooke, M.D., Ph.D.
$660,000 for 2 years

New Chemokine-Derived Therapeutics Targeting Stem Cell Migration
Burnham Institute for Medical Research
P.I.: Ziwei Huang, Ph.D.
$760,000 for 2 years
Also referenced in: Spinal Cord Injury, Traumatic Brain Injury

COMPREHENSIVE RESEARCH GRANT PROGRAM

Epigenetic gene regulation during the differentiation of human embryonic stem cells: Impact on neural repair
University of California, Los Angeles
P.I.: Guoping Fan, Ph.D.
$2,520,000 for 4 years

MEF2C-Directed Neurogenesis From Human Embryonic Stem Cells
Burnham Institute for Medical Research
P.I.: Stuart A. Lipton, M.D., Ph.D.
$3,040,000 for 4 years
Also referenced in: Parkinson's Disease

Human stem cell derived oligodendrocytes for treatment of stroke and MS
University of California, San Francisco
P.I.: Samuel J. Pleasure, M.D., Ph.D.
$2,570,000 for 4 years
Also referenced in: Multiple Sclerosis

TRAUMATIC BRAIN INJURY (TBI)

LEON J. THAL SEED GRANT PROGRAM

New Chemokine-Derived Therapeutics Targeting Stem Cell Migration
Burnham Institute for Medical Research
P.I.: Ziwei Huang, Ph.D.
$760,000 for 2 years
Also referenced in: Spinal Cord Injury, Stroke

Dollar amount shown is the amount requested rounded to the nearest $5,000, not the amount awarded.

ABSTRACTS

[Provided by applicant]

SPINAL CORD INJURY

The Immunological Niche: Effect of immunosuppressant drugs on stem cell proliferation, gene expression, and differentiation in a model of spinal cord injury.

University of California, Irvine
Principal Investigator: Brian John Cummings, Ph.D. Leon J. Thal SEED Grant Program

Our understanding of the effect of immunosuppressive agents on stem cell proliferation and differentiation in the central nervous system is limited. Indeed, even the necessity for long-term immunosuppression to promote the survival of stem cells grafted into the "immunoprivileged" central nervous system (CNS) is unknown. Grafting multipotent stem cells into the injured CNS often results in a failure of the cells to survive. If the cells survive, often they differentiate into astrocytes, a cell-type not considered beneficial. We recently grafted human stem cells (hCNS-SC) into spinal injured mice and observed behavioral improvements coupled with differentiation of these human cells into neurons and oligodendrocytes. We also observed mouse-human synapse formation and remyelination. The mice we used lacked a functional immune system, enabling us to grafting human cells into the mice without the use of immunosuppressants. When these same cells were grafted into spinal injured rats with a normal immune system, we had to immunosuppress the animals. Exposure of these human stem cells to immunosuppressive drugs resulted poor cell survival. The cells that did survive predominantly differentiated into astrocytes. Did the immunosuppressive drugs we used alter the ability of the human stem cells to differentiate into useful cells?

All cell-based therapeutic approaches are dependent upon either immunosuppression in an otherwise normal animal or testing for proof of principal in an immunodeficient animal model. This has quite significant implications for animal experiments or human trials, where continuous immunosuppression is required to obtain successful graft survival. No one knows if there are direct effects of immunosuppressant drugs on neural stem cells.

Stem cells may also respond differently to immunosuppression depending on their "ontogenetic" age (embryonic vs. fetal vs. adult). There is a common perception that "young" ES cells will have greater potential than "older" stem cells. Stem cells isolated at different ontogenetic stages might respond differently to immunosuppression.

We predict that the immunosuppressive drugs will exert direct effects on stem cell proliferation, gene expression, and fate determination, both in cell culture and when grafted into animals with spinal cord injury. We will also test if "ontogenetic" age alters the responsiveness of stem cells.

New Chemokine-Derived Therapeutics Targeting Stem Cell Migration

Burnham Institute for Medical Research
Principal Investigator: Ziwei Huang, Ph.D. Leon J. Thal SEED Grant Program
Also referenced in: Stroke, Traumatic Brain Injury (TBI)

This proposal describes a sharply-focused, timely, and rigorous effort to develop new therapies for the treatment of injuries of the Central Nervous System (CNS). The underlying hypothesis for this proposal is that chemokines and their receptors (particularly those involved in inflammatory cascades) actually play important roles in mediating the directed migration of human neural stem cells (hNSCs) to, as well as engagement and interaction with, sites of CNS injury, and that understanding and manipulating the

molecular mechanism of chemokine-mediated stem cell homing and engagement will lead to new, better targeted, more specific, and more efficacious chemokine-mediated stem cell-based repair strategies for CNS injury. In recent preliminary studies, we have discovered and demonstrated the important role of chemokine SDF-1-alpha and its receptor CXCR4 in mediating the directed migration of hNSCs to sites of CNS injury. To manipulate this SDF-1-alpha/CXCR4 pathway in stem cell migration, we have developed Synthetically and Modularly Modified Chemokines (SMM-chemokines) as highly potent and specific therapeutic leads. Here in this renewal application we propose to extend our research into a new area of stem cell biology and medicine involving chemokine receptors such as CXCR4 and its ligand SDF-1. Specifically, we will design more potent and specific analogs of SDF-1-alpha to direct the migration of beneficial stem cells toward the injury sites for the repair process.

Genetic manipulation of human embryonic stem cells and its application in studying CNS development and repair

University of California, San Diego
Principal Investigator: Binhai Zheng, Ph.D. Leon J. Thal SEED Grant Program
Also referenced in: Amyotrophic lateral sclerosis (ALS) /Spinal Muscular Atrophy (SMA)

The advent of human embryonic stem cells (hESCs) has offered enormous potential for regenerative medicine and for basic understanding of human biology. On the one hand, hESCs can be turned into many different cell types in culture dish, and specific cell types derived from hESCs offer an almost infinite source for cellular replacement therapies. This is the primary reason for which hESCs have received much attention from the general public. On the other hand, scientists can study the properties of hESCs and their derivatives, and determine the effect of genes and molecules on such properties either in culture dish or with transplantation studies in live animals. This second aspect of hESC research would not only significantly enhance our understanding of the function of human genes, but will greatly augment our ability to apply hESCs in transplantation therapies and regenerative medicine. To attain the full potential of hESCs, genetic manipulation of hESCs is essential. In this proposal, we will establish the methods to genetically manipulate an increasingly used, non-federally approved hESC line, the HUES-9, and assess the feasibility to use genetically modified HUES-9 cells in cell transplantation studies to assess the integration of hESCs into the mouse central nervous system. We propose to achieve both homologous recombination (i.e. gene targeting) and transgene expression (with bacterial artificial chromosome), which have complementary utilities in assaying gene function in addition to the opportunity to label hESCs or their derivatives with fluorescent markers. Specifically, with genetic engineering of hESCs we will be able to 1) label hESCs and specific cell types derived from hESCs so that they can be readily followed in culture dish and in animals that have received cellular transplants; 2) disturb an endogenous gene or add more copies of a gene so that the effect of a gene of interest can be assessed (for this purpose, a gene involved in the development of a major motor tract, the corticospinal tract, will be studied). We will then transplant genetically engineered hESCs and their derivatives into the embryonic and adult mouse CNS to assess how well these cells integrate into the mouse CNS, and whether such transplanted animals can serve as valid models to study the effect of genes on hESC function in live animals. In transplantation studies involving adult mouse recipients, injured mouse CNS will be used in addition to intact CNS in order to evaluate the potential of hESCs to integrate into injured CNS, which has direct implications on the therapeutic potential of these cells. In summary, our proposal will establish the methods and tools to genetically manipulate HUES-9 cells, explore a paradigm to study human genes and cells in a context of neural development and cellular therapies, and will pave the way for future studies of genes and pathways in basic biology and regenerative medicine with hESCs.

hESC-Derived Motor Neurons For the Treatment of Cervical Spinal Cord Injury
University of California, Irvine
Principal Investigator: Hans S. Keirstead, Ph.D. Comprehensive Research Grant Program

Cervical spinal cord injuries result in a loss of upper limb function because the cells within the spinal cord that control upper limb muscles are destroyed. The goal of this research program is to create a renewable human source of these cells, to restore upper limb function in both acute and chronic spinal cord injuries. There are two primary challenges to the realization of this goal: 1) a source of these human cells in high purity, and 2) functional integration of these cells in the body after transplantation.

Human embryonic stem cells (hESCs) can form any cell in the body, and can reproduce themselves almost indefinitely to generate large quantities of human tissue. One of the greatest challenges of hESC research is to find ways to restrict hESCs such that they generate large amounts on only one cell type in high purity such that they could be used to replace lost cells in disease or trauma. Our laboratory was the first laboratory in the world to develop a method to restrict hESCs such that they generate large amounts of only one cell type in high purity. That cell type is called an oligodendrocyte, which insulates connections in the spinal cord to allow them to conduct electricity. Transplantation of these cells was useful for treating spinal cord injuries in rats if the treatment was given one week after the injury. That treatment is being developed for use in humans.

Recent studies in our laboratory indicate that we have succeeded in restricting hESCs to generate large quantities of a different cell type in the spinal cord, that which controls upper limb muscles. We have generated large quantities of these human cells, grown them with human muscle, and demonstrated that they connect and control the human muscle. The cells also express markers that are appropriate for this cell type.

Here we propose to generate these cells in high purity from hESCs and genetically modify them so that they can be induced to grow over inhibitory environments that exist in the injured spinal cord. We will then determine whether these human cells have the ability to regenerate the injured tissue in the spinal cord, and restore lost function. All of our studies will be conducted in an FDA-compliant manner, which will speed the translation of our results to humans if we are successful. The studies outlined in this proposal represent a novel approach to treating spinal cord injury, which might work for both acute and chronic injuries.

Spinal ischemic paraplegia: modulation by human embryonic stem cell implant.
University of California, San Diego
Principal Investigator: Martin Marsala, M.D. Comprehensive Research Grant Program

Ischemia-induced paraplegia often combined with a qualitatively defined increase in muscle tone (i.e. spasticity and rigidity) is a serious complication associated with a temporary aortic cross-clamping (a surgical procedure to repair an aortic aneurysm).

In addition to spinal ischemic injury-induced spasticity and rigidity a significant population of patients with traumatic spinal injury develop a comparable qualitative deficit i.e. debilitating muscle spasticity. At present there are no effective treatment which would lead to a permanent amelioration of spasticity and rigidity and corresponding improvement in ambulatory function. In recent studies, by using rat model of spinal ischemic injury we have demonstrated that spinal transplantation of rat or human neurons leads to a clinically relevant improvement in motor function and correlates with a long term survival and maturation of grafted cells. More recently we have demonstrated a comparable maturation of human

spinal precursors grafted spinally in immunosupressed minipig. In the proposed set of experiments we wish to characterize a therapeutical potential of human blastocyst-derived neuronal precursors when grafted into previously ischemia- injured rat or minipig spinal cord. Defining the potency of spinally grafted hESC-derived neuronal precursors in two in vivo models of spinal ischemic injury serves to delineate the differences and/or uniformity in the cell maturation when cells are transplanted in 2 different animals species and can provide an important data set for future implications of such a therapies in human patients.

STROKE

EC regeneration in cerebrovascular ischemia: role of NO

Stanford University

Principal Investigator: John P. Cooke, M.D., Ph.D. Leon J. Thal SEED Grant Program

Stroke is the third leading cause of death and the leading cause of disability in this country, affecting about 650,000 people in the US each year. Currently approved therapies for stroke are directed toward acutely restoring blood flow (using drugs that break up clot). A new approach is to use stem cells to regenerate portions of the brain that are damaged in a stroke. Stem cells can be obtained from adult individuals, or from embryos. Studies using adult stem cells have shown that only a small fraction of these cells are capable of transforming into brain cells. Another problem is that in patients with stroke, many different types of brain cells must be replaced. Furthermore, the replacement cells must reconstitute the normal architecture of the lost brain. Additionally, the stem cells must overcome the hostile metabolic milieu in the ischemic brain, which includes poor blood flow, as well as the adverse metabolic environment that caused the stroke in the first place (eg. high blood sugar, high cholesterol, high blood pressure).

So in this proposal we are taking a different approach. We will develop methods to make blood vessels using human embryonic stem cells (HESC). HESC derived blood vessel cells will be injected into rats that have had a surgically-induced stroke. We will determine if the HESC-derived vascular cells find their way to the area of poor blood flow. We will determine if these cells survive and if they generate new blood vessels.

In other works, rather than attempting to provide stem cells that will develop into complex brain tissue after stroke, we intend to first restore the brain vessels in the area of the stroke. We hypothesize that "if we build the road, they will come", ie. the restoration of the brain vessels will enhance survival of brain tissue in areas of poor flow, and may induce repair of the injured area, by encouraging neighboring nerve cells to migrate into the area.

Furthermore, we plan to genetically engineer the HESCs to make them hardier. The area of stroke in the brain is a hostile environment for cells. One of the factors that mediates the adverse metabolic effects is a substance called ADMA (asymmetric dimethylarginine). We will engineer HESC that are more able to handle this substance, and determine if that genetic modification gives the HESC a better chance of surviving and forming blood vessels.

This proposal will provide insights into the use of human embryonic stem cells (HESC) for regenerating the injured brain after a stroke.

New Chemokine-Derived Therapeutics Targeting Stem Cell Migration
Burnham Institute for Medical Research
Principal Investigator: Ziwei Huang, Ph.D. Leon J. Thal SEED Grant Program
Also referenced in: Traumatic Brain Injury, Spinal Cord Injury

This proposal describes a sharply-focused, timely, and rigorous effort to develop new therapies for the treatment of injuries of the Central Nervous System (CNS). The underlying hypothesis for this proposal is that chemokines and their receptors (particularly those involved in inflammatory cascades) actually play important roles in mediating the directed migration of human neural stem cells (hNSCs) to, as well as engagement and interaction with, sites of CNS injury, and that understanding and manipulating the molecular mechanism of chemokine-mediated stem cell homing and engagement will lead to new, better targeted, more specific, and more efficacious chemokine-mediated stem cell-based repair strategies for CNS injury. In recent preliminary studies, we have discovered and demonstrated the important role of chemokine SDF-1-alpha and its receptor CXCR4 in mediating the directed migration of hNSCs to sites of CNS injury. To manipulate this SDF-1-alpha/CXCR4 pathway in stem cell migration, we have developed Synthetically and Modularly Modified Chemokines (SMM-chemokines) as highly potent and specific therapeutic leads. Here in this renewal application we propose to extend our research into a new area of stem cell biology and medicine involving chemokine receptors such as CXCR4 and its ligand SDF-1. Specifically, we will design more potent and specific analogs of SDF-1-alpha to direct the migration of beneficial stem cells toward the injury sites for the repair process.

Epigenetic gene regulation during the differentiation of human embryonic stem cells: Impact on neural repair
University of California, Los Angeles
Principal Investigator: Guoping Fan, Ph.D. Comprehensive Research Grant Program

Human embryonic stem cells (hESCs) have the potential to become all sorts of cells in human body including nerve cells. Moreover, hESCs can be expanded in culture plates into a large quantity, thus serving as an ideal source for cell transplantation in clinical use. However, the existing hESC lines are not fully characterized in terms of their potential to become specific cell types such as nerve cells. It is also unclear if the nerve cells that are derived from hESCs are totally normal when tested in cell transplantation experiments. One of the goals for our proposal is to compare the quality and the potential of eight lines of hESCs in their capacity to become nerve cells. To measure if the nerve cells that are derived from hESCs are normal when compared to the nerve cells in normal human beings, we will examine the levels of gene expression and the mechanisms that control gene expression in hESC-derived nerve cells. Specifically, we will examine the pattern of DNA modification, namely DNA methylation, in the DNA of nerve cells. This DNA modification is involved in the inhibition of gene expression. It is known that if DNA methylation pattern is abnormal, it can lead to human diseases including cancer and mental retardation disorders. We will use a DNA microarray technology to identify DNA methylation pattern in the critical regions where gene expression is controlled. Our recent results suggest that increased DNA methylation is observed in hESC-derived nerve cells. In this proposal, we will also test if we can balance the level of DNA methylation through pharmacological treatment of enzymes that are responsible for DNA methylation. Finally, we will test if hESC-derived nerve cells can repair the brain after injury . A mouse stroke model will be used for testing the mechanisms stem cell-mediated repair and recovery in the injured brain and for selecting the best nerve cells for cell transplantation. Our study will pave the way for the future use of hESC-derived nerve cells in clinical treatment of nerve injury and neurodegenerative diseases such as stroke and Parkinson's disease.

MEF2C-Directed Neurogenesis From Human Embryonic Stem Cells
Burnham Institute for Medical Research
Principal Investigator: Stuart A. Lipton, M.D., Ph.D. Comprehensive Research Grant Program
Also referenced in: Parkinson's Disease

Understanding differentiation of human embryonic stem cells (hESCs) provides insight into early human development and will help directing hESC differentiation for future cell-based therapies of Parkinson's disease, stroke and other neurodegenerative conditions.

The PI's laboratory was the first to clone and characterize the transcription factor MEF2C, a protein that can direct the orchestra of genes to produce a particular type of cell, in this case a nerve cell (or neuron). We have demonstrated that MEF2C directs the differentiation of mouse ES cells into neurons and suppresses glial fate. MEF2C also helps keep new nerve cells alive, which is very helpful for their successful transplantation. However, little is known about the role of MEF2C in human neurogenesis, that is, its ability to direct hESC differentiation into neuronal lineages such as dopaminergic neurons to treat Parkinson's disease and its therapeutic potential to promote the generation of nerve cells in stem cell transplantation experiments. The goal of this application is to fill these gaps.

The co-PI's laboratory has recently developed a unique procedure for the efficient differentiation of hESCs into a uniform population of neural precursor cells (NPCs), which are progenitor cells that develop from embryonic stem cells and can form different kinds of mature cells in the nervous system. Here, we will investigate if MEF2C can instruct hESC-derived NPCs to differentiate into nerve cells, including dopaminergic nerve cells for Parkinson's disease or other types of neurons that are lost after a stroke. Moreover, we will transplant hESC-NPCs engineered with MEF2C to try to treat animal models of stroke and Parkinson's disease. We will characterize known and novel MEF2C target genes to identify critical components in the MEF2C transcriptional network in the clinically relevant cell population of hESC-derived neural precursor cells (hESC-NPCs).

Specifically we will: 1) determine the function of MEF2C during in vitro neurogenesis (generation of new nerve cells) from hESC-NPCs; 2) investigate the therapeutic potential of MEF2C engineered hESC-NPCs in Parkinson's and stroke models; 3) determine the MEF2C DNA (gene) binding sites and perform a "network" analysis of MEF2C target genes in order to understand how MEF2C works in driving the formation of new nerve cells from hESCs.

Human stem cell derived oligodendrocytes for treatment of stroke and MS
University of California, San Francisco
Principal Investigator: Samuel J. Pleasure, M.D., Ph.D. Comprehensive Research Program
Also referenced in: Multiple Sclerosis

Strokes that affect the nerves cells, i.e., 'gray matter', consistently receive the most attention. However, the kind of strokes that affecting the 'wiring' of the brain, i.e., 'white matter', cause nearly as much disability. The most severe disability is caused when the stroke is in the wiring (axons) that connect the brain and spinal cord; as many as 150,000 patients are disabled per year in the US from this type of stroke. Although oligodendrocytes ('oligos' are the white matter cells that produce the lipid rich axonal insulator called myelin) are preferentially damaged during these events, stem cell-derived oligos have not been tested for their efficacy in preclinical (animal) trials. These same white matter tracts (located underneath the gray matter, called subcortical) are also the primary sites of injury in MS, where multifocal inflammatory attack is responsible for stripping the insulating myelin sheaths from axons resulting in axonal dysfunction and degeneration. Attempts to treat MS-like lesions in animals using undifferentiated stem cell transplants are promising, but most evidence suggests that these approaches work by changing the inflammation response (immunomodulation) rather than myelin regeneration. While

immunomodulation is unlikely to be sufficient to treat the disease completely, MS may not be amenable to localized oligo transplantation since it is such a multifocal process. This has led to new emphasis on approaches designed to maximize the response of endogenous oligo precursors that may be able to regenerate myelin if stimulated. We hypothesize that by exploiting novel features of oligo differentiation in vitro (that we have discovered and that are described in our preliminary data) that we will be able to improve our ability to generate oligo lineage cells from human embryonic stem cells and neural stem cells for transplantation, and also to develop approaches to maximize oligo development from endogenous precursors at the site of injury in the brain. This proposal will build on our recent successes in driving oligo precursor production from multipotential mouse neural stem cells by expressing regulatory transcription factors, and apply this approach to human embryonic and neural stem cells to produce cells that will be tested for their ability to ameliorate brain damage in rodent models of human stroke. Furthermore, we hope to develop approaches that may facilitate endogenous recruitment of oligo precursors to produce mature oligos, which may prove a viable regenerative approach to treat a variety of white matter diseases including MS and stroke.

TRAUMATIC BRAIN INJURY

New Chemokine-Derived Therapeutics Targeting Stem Cell Migration

Burnham Institute for Medical Research
Principal Investigator: Ziwei Huang, Ph.D. Leon J. Thal SEED Grant Program
Also referenced in: Spinal Cord Injury, Stroke

This proposal describes a sharply-focused, timely, and rigorous effort to develop new therapies for the treatment of injuries of the Central Nervous System (CNS). The underlying hypothesis for this proposal is that chemokines and their receptors (particularly those involved in inflammatory cascades) actually play important roles in mediating the directed migration of human neural stem cells (hNSCs) to, as well as engagement and interaction with, sites of CNS injury, and that understanding and manipulating the molecular mechanism of chemokine-mediated stem cell homing and engagement will lead to new, better targeted, more specific, and more efficacious chemokine-mediated stem cell-based repair strategies for CNS injury. In recent preliminary studies, we have discovered and demonstrated the important role of chemokine SDF-1-alpha and its receptor CXCR4 in mediating the directed migration of hNSCs to sites of CNS injury. To manipulate this SDF-1-alpha/CXCR4 pathway in stem cell migration, we have developed Synthetically and Modularly Modified Chemokines (SMM-chemokines) as highly potent and specific therapeutic leads. Here in this renewal application we propose to extend our research into a new area of stem cell biology and medicine involving chemokine receptors such as CXCR4 and its ligand SDF-1. Specifically, we will design more potent and specific analogs of SDF-1-alpha to direct the migration of beneficial stem cells toward the injury sites for the repair process.

APPROVED APPLICATIONS RELATED TO THE BIOLOGY OF HUMAN EMBRYONIC STEM CELLS

SELF-RENEWAL

Embryonic stem cells have the unique ability to replace themselves continuously (self-renew), and at the same time have the capacity to give rise to all the different cell types in the body. Past studies using mouse embryonic stem cells and adult human stem cells provided insights into possible molecular pathways for accomplishing the remarkable feats of continuous self-renewal and specific differentiation. Candidates for controlling self-renewal include regulatory RNA molecules (microRNAs), chemical modifications of DNA and/or DNA binding proteins (histones), key growth factors and their signaling proteins, and transcription factors, which are regulatory proteins that bind to DNA and control expression of genes. While previous studies using animal and adult stem cells identified some proteins that regulate self-renewal, the key players may be different in stem cells derived from the human blastocyst. In 2007, 12 awards are approved for research that delves into the detailed molecular events responsible for self-renewal of human embryonic stem cells. Lessons learned from this research will help optimize the growth and maintenance of healthy human embryonic stem cells for use in regenerative medicine.

LEON J. THAL SEED GRANT PROGRAM

MicroRNA Regulation of Human Embryonic Stem Cell Self-Renewal and Differentiation
University of California, San Francisco
P.I.: Robert Hector Blelloch, M.D., Ph.D.
$635,000 for 2 years

Regulation of Specific Chromosomal Boundary Elements by CTCF Protein Complexes in Human Embryonic Stem Cells
The Salk Institute for Biological Studies
P.I.: Beverly M. Emerson, Ph.D.
$680,000 for 2 years

Assessing the role of Eph/ephrin signaling in hESC growth and differentiation
University of California, Santa Cruz
P.I.: David Feldheim, Ph.D.
$500,000 for 2 years

Cellular epigenetic diversity as a blueprint for defining the identity and functional potential of human embryonic stem cells
University of California, Los Angeles
P.I.: Siavash K. Kurdistani, M.D.
$645,000 for 2 years

Stem Cell Survival and Differentiation Through Chemical Genetics
University of California, Riverside
P.I.: Michael C. Pirrung, Ph.D.
$545,000 for 2 years

Transcriptional Regulation of Human Embryonic Stem Cells
University of California, San Francisco
P.I.: Miguel Ramalho-Santos, Ph.D.
$620,000 for 2 years

Dollar amount shown is the amount requested rounded to the nearest $5,000, not the amount awarded.

A Chemical Approach to Stem Cell Biology
Scripps Research Institute
P.I.: Peter G. Schultz, Ph.D.
$785,000 for 2 years

Role of the tumor suppressor gene, p16INK4a, in regulating stem cell phenotypes in embryonic stem cells and human epithelial cells
University of California, San Francisco
P.I.: Thea D. Tlsty, Ph.D.
$640,000 for 2 years
Also referenced in: Genomic Instability and Cancer

Role of HDAC in human stem cells pluripotentiality and differentiation
The J. David Gladstone Institutes
P.I.: Eric M. Verdin, M.D.
$795,000 for 2 years

Self-renewal of human embryonic stem cells
University of Southern California
P.I.: Qilong Ying, Ph.D.
$665,000 for 2 years

COMPREHENSIVE RESEARCH GRANT PROGRAM

Improved hES Cell Growth and Differentiation
University of California, Irvine
P.I.: Peter Donovan, Ph.D.
$2,510,000 for 4 years

Mechanisms to maintain the self-renewal and genetic stability of human embryonic stem cells
University of California, San Diego
P.I.: Yang Xu, Ph.D.
$2,570,000 for 4 years
Also referenced in: Genomic Instability and Cancer

Dollar amount shown is the amount requested rounded to the nearest $5,000, not the amount awarded.

ABSTRACTS

[Provided by applicant]

SELF-RENEWAL

MicroRNA Regulation of Human Embryonic Stem Cell Self-Renewal and Differentiation

University of California, San Francisco
Principal Investigator: Robert Hector Blelloch, M.D., Ph.D. Leon J. Thal SEED Grant Program

A major hurdle for regenerative medicine is the safe transplantation of human embryonic stem (ES) cells or their derivatives into patients. While the unlimited growth potential of ES cells is a major asset for their potential in tissue replacement, it is also a major risk for tumorigenesis. Therefore, it is critical to determine what molecules are responsible for silencing the tumorigenic risk of embryonic stem cell derivatives as occurs during the process of normal development. Identification of such molecules should provide both markers for tumorigenic risk as well as potential targets for therapeutic intervention when tumors do develop from transplanted tissue. We now know that most, if not all adult cells can revert to an early stem cell phenotype. This has been proven by a technique called somatic cell nuclear transfer, where adult cell nuclei are transferred into oocytes and allowed to develop as early embryos. These embryos reactivate the embryonic stem cell program within the adult nuclei. Cells derived from these embryos, the embryonic stem cells, have regained the ability to proliferate indefinitely, a property termed self-renewal. Therefore, considering there is in the order of one hundred trillion cells in the adult human, it is amazing that some fraction of cells do not commonly reactivate the stem cell program by chance. This is because human have evolved an amazing and complex network of molecules whose main purpose is to permanently silence critical components of the embryonic stem cell program. Unfortunately, this network is poorly understood. The aim of our laboratory and of this research proposal is to identify these factors and use them to our advantage. We have discovered in the mouse model that small RNA molecules called microRNAs are essential for the silencing of the embryonic stem cell's capacity to self-renew. In this grant, we propose to confirm a similar role for microRNAs in human ES cell differentiation. Furthermore, we propose to identify the molecular nature of the specific microRNAs responsible for this function. These miRNAs could then be used as markers for both the developmental potential of embryonic stem cells derived by various means as well as a marker for the appropriate silencing of self-renewal in ES cell derived tissues prior to transplant in patients. Furthermore, they could provide targets for therapeutic intervention in the unfortunate scenario of tumor formation from transplanted tissues that had failed to fully silence the stem cell program.

Regulation of Specific Chromosomal Boundary Elements by CTCF Protein Complexes in Human Embryonic Stem Cells

The Salk Institute for Biological Studies
Principal Investigator: Beverly M. Emerson, Ph.D. Leon J. Thal SEED Grant Program

The genetic information contained in all human cells is arranged into distinct territories or 'neighborhoods' with barriers or 'fences' that protect the action in one neighborhood from spilling over into an adjacent region. In this way, one gene (A) can be working while its neighboring genes (B and C) are resting. As physiological conditions change in the body, appropriate signals are transmitted to cells that instruct genes to alter their genetic 'programming' by opening or closing the fences. This allows gene A to be turned off and genes B and C to start working. Importantly, these 'fences' can control large numbers of genes that regulate critical cellular processes. For example, a well-known fence borders a chromosomal region containing genes that encode oxygen-carrying hemoglobin. By opening or closing this fence, hemoglobin synthesis, and our oxygen carrying ability, can be turned on or off. Many, as yet, unidentified fences are likely to exist in our genetic material. This proposal is designed to find the fence(s) that border certain genes (Nanog-Stellar-GDF3) that are important to maintain stem cells in their most plastic state

that is, having the ability to become any other cell type. Once we identify the borders/fences of this chromosomal region, we plan to investigate how they are themselves switched on or off. This switch is very likely to depend upon specific proteins that interact with the fences or borders and serve as 'latches' to keep the gates open or closed and the Nanog gene working or resting. Information about the exact proteins or 'latches' that control the Nanog neighborhood will enable us to begin to devise strategies, through genetics or pharmacological means, to open or close this particular fence at will and regulate the activity of the Nanog gene. The ability to maintain an active Nanog gene may facilitate stem cell self-renewal or reprogram adult somatic cells to progenitors that are more easily directed to another cell type. By contrast, the capacity to turn off the Nanog gene may be important for the treatment of stem cells that have acquired tumorigenic potential through persistent Nanog expression and inappropriate self-renewal. In the larger scope, information from this proposal may serve as a platform by which unique proteins that control other fences can be identified. Pharmacological manipulation of these unique proteins may selectively control the activity of chromosomal neighborhoods that specify distinct cell fates.

Assessing the role of Eph/ephrin signaling in hESC growth and differentiation
University of California, Santa Cruz
Principal Investigator: David Feldheim, Ph.D. Leon J. Thal SEED Grant Program

An important aspect of understanding stem cell biology is to have a basic understanding of the processes that balance stem cell self-renewal and differentiation. Stem cell proliferation and differentiation signals are at least partially regulated by direct contact between cells. For example, stem cells normally reside in a specific microenvironment, or "niche", that integrates specific cell-cell contacts in order to translate information from the environment into proliferation patterns.

In this SEED proposal we plan to investigate the role of Eph/ephrin signaling in hESC growth and differentiation. Eph receptor tyrosine kinases and their ligands, ephrins, are large gene families that initiate signal transduction pathways which lead to changes in cellular adhesion, proliferation, and migration. Both Ephs and ephrins are expressed on the surfaces of cells, thus restricting their interactions to sites of direct cell-cell contact. It is known that Ephs and ephrins are expressed in hESCs and are therefore in the right place to be involved in regulating hESC proliferation and differentiation decisions. To better understand how Ephs and ephrins might be involved in hESC growth regulation, we plan to characterize the expression of Ephs and ephrins in hESCs during different stages of growth and neural differentiation to determine if Eph/ephrin signaling is used to regulate proliferation and differentiation of hESCs.

The characterization of the role that Ephs and ephrins play in hESCs will provide insights into how stem cell proliferation is regulated in culture and will likely be applicable to how stem cell niches are organized in vivo. This understanding may allow for the development of standard culture conditions that will optimize both self-renewal and homogeneity of cells. This will in turn lead to more efficient large-scale production of stem cell populations and also methods for maintaining a self-renewing state in culture. Conversely, in a therapeutic setting, even a small number of undifferentiated cells could result in tumor formation; therefore, we also need to understand how to prevent self-renewal of stem cells.

Cellular epigenetic diversity as a blueprint for defining the identity and functional potential of human embryonic stem cells
University of California, Los Angeles
Principal Investigator: Siavash K. Kurdistani, M.D. Leon J. Thal SEED Grant Program

Human embryonic stem (ES) cells have the capacity to self-renew but also give rise to other cell types. How this capacity is regulated and what factors determine one fate over another is an active area of research. This is because by understanding the decision making process the a stem cell goes through,

we might be able to manipulate the process and make stem cells generate more of themselves or other cell types of interest. Preliminary studies indicate that one important determinant of stem cell fate is its "epigenetic" information content.

In humans, DNA is tightly wrapped around a core of proteins called histones to form chromatin-the physiologically relevant form of the genome. The histones can be modified by small chemical molecules which can affect the structure of chromatin, allowing for a level of control on gene expression. The patterns of occurrences of the histone modifications throughout chromatin is highly regulated and can increase the capacity of the genome to store and process biological information beyond the DNA sequence. This information which is heritable but not encoded in the sequence of DNA is referred to as "epigenetics." The modifications of histones, therefore, contribute to the epigenetic information content of a human ES cell.

We have found that individual ES cells from mouse have different patterns of histone modifications, and thus, different epigenetic information content. We believe these differences may affect the fate decisions of stem cells. If so, then the histone modifications may act as a natural indicator of the potential of ES cells to make certain fate decisions. The histone modifications may also provide a natural tool by which cell fate decisions can be influenced. In this proposal, we intend to determine the epigenetic information of content of several human ES cell lines and relate that information to the potential of cells to make self-renewal versus differentiation decisions. Our work will provide a fast and high-throughput measure by which appropriate ES cells can be chosen for a clinical application of interest.

Stem Cell Survival and Differentiation Through Chemical Genetics
University of California, Riverside
Principal Investigator: Michael C. Pirrung, Ph.D. Leon J. Thal SEED Grant Program

This project will test the effects of chemical compounds similar to conventional pills for their abilities to keep human embryonic stem cells growing and multiplying in the laboratory or to help them become one of the specialized types of cells, like spinal cord cells, found in the human body. Many of the substances currently used to accomplish these goals come from animals or animal cells. They carry a risk of transmitting diseases or making the human cells display some animal traits, either of which would make cells derived from human stem cells useless for transplantation and regenerative medicine. These animal-derived substances can also be very costly. Replacing these expensive materials will be essential to the eventual development of therapies for patients. These will be basic studies using one of the already-approved human embryonic stem cell lines. However, the molecules that are prepared in this work and discovered to have desirable properties should be applicable to human embryonic stem cell lines derived in the future by any technically and ethically appropriate method. The project therefore aims to discover new tools for embryonic stem cell research that will be useful to develop human therapies.

Transcriptional Regulation of Human Embryonic Stem Cells
University of California, San Francisco
Principal Investigator: Miguel Ramalho-Santos, Ph.D. Leon J. Thal SEED Grant Program

Embryonic Stem (ES) cells can be grown indefinitely in the lab and can be turned into any cell type of the human body. Because of these properties, it may one day be possible to use ES cells to generate cell types in the lab that can then be transplanted into patients that need them. This approach may provide new treatments for devastating and presently incurable conditions such as type I diabetes, Parkinson's disease, muscular dystrophies, spinal cord injuries, and many others. However, before human ES cells can safely be used in the clinic, it will be essential to understand how they function. For example, if rapid cell division is not kept in check in ES cells, they can give rise to tumors upon transplantation. Our proposal is directly aimed at understanding the genetic regulation of human ES cells.

We developed a very innovative approach to understand how gene activity is regulated in human ES cells. Our very significant progress so far it can be summarized as follows:

- we identified the genes that are preferentially activated in ES cells;
- we discovered several DNA sequences that act as genetic switches to turn ES cell genes on or off;
- we identified an operator protein that activates one of these switches;
- we discovered that this protein is essential to maintain rapid cell division of mouse ES cells.

We now propose to investigate the function of this protein in human ES cells. We further propose to identify other operator proteins that activate genetic switches revealed by our work. Our ultimate goal is to identify all the operator proteins, the corresponding genetic switches, and their combined mode of action in human ES cells.

We expect that this research will make the following significant contributions:
1. Our research may lead to the development of diagnostic tests that detect the activity of the operator proteins and genetic switches that we have identified. These diagnostic tests may be important tools for quality control of human ES cells;
2. The operator proteins identified are expected to be critical regulators of rapid cell division of human ES cells. Understanding what those operator proteins are may lead to the development of new drugs to prevent the formation of tumors upon transplantation of ES cells;
3. The current methods to obtain a particular cell type from ES cells still result in a mixture of different cell types. If we understand how genes are activated in ES cells, we may be able to turn on the precise set of genes that leads to the formation of a particular cell type of interest, and thus obtain pure populations of cells needed by patients;
4. If we understand what are the essential operator proteins that regulate gene activity in ES cells, we may be able to formulate a cocktail of these proteins that is capable of resetting the genetic program of a patient's own cells back to that of ES cells. This way the transplanted cells will be immune-matched to the patient, and therefore will not be rejected.

A Chemical Approach to Stem Cell Biology
Scripps Research Institute
Principal Investigator: Peter G. Schultz, Ph.D. Leon J. Thal SEED Grant Program

The aim of this project is to screen large collections of small molecules to identify molecules that allow one to propagate human embryonic stem cells (hESCs) in cell culture under defined conditions in an undifferentiated, pluripotent state. The chemical structures of any biologically active small molecules will be optimized with respect to potency, selectivity and biological stability. The ability of hESCs proliferated in the presence of such small molecules to be differentiated into specific cell lineages both in cell culture and in vivo will also be assessed. And finally, we will determine the mechanism of action of active small molecules by a variety of biochemical and genomic methods. The demonstration that one can identify synthetic drug-like molecules that allow one to control the self-renewal and/or differentiation of hESCs will represent an important step in the ultimate therapeutic application of hESCs to human disease. In addition, biological studies of such molecules should provide new insights into the processes that control stem cell biology.

Role of the tumor suppressor gene, p16INK4a, in regulating stem cell phenotypes in embryonic stem cells and human epithelial cells

University of California, San Francisco
Principal Investigator: Thea D. Tlsty, Ph.D. Leon J. Thal SEED Grant Program

Also referenced in: Genomic Instability and Cancer

The roles of stem cells are to generate the organs of the body during development and to stand ready to repair those organs through repopulation after injury. In some cases these properties are not correctly regulated and cells with stem cell properties expand in number. Recent work is demonstrating that the genes that control stem cell properties are sometimes the same genes that are mutated in cancer. This means that a cell can simultaneously acquire stem cell properties and cancer properties. In order to effectively use stem cells for therapeutic purposes we need to understand the link between these two programs and devise ways to access one program without turning on the other. In other words, we would like to expand stem cell populations without them turning into cancer.

Recent work in our laboratory has found that the reduction of a specific tumor suppressor gene, p16, not only removes an important barrier to cancer but also confers stem cell properties within the cell. Cells that have reduced p16 activity can turn on a program that increases and reduces expression of specific genes that control differentiation. In this proposal we will test whether the continued reduction of this tumor suppressor gene creates human embryonic stem cells (hESC) that are unable to differentiate. We hypothesize that the lack of p16 represses multi-lineage potential by activating an epigenetic program and silencing genes that drive differentiation. To test this hypothesis we will first determine if lack of p16 activity is necessary for hESCs to develop into different cell types. Second, we will determine if continued lack of p16 activity is sufficient to inhibit differentiation of hESCs. Finally, we will determine if transient lack of p16 activity is sufficient for a non-stem cell to exhibit properties of a stem cell after propagation in a stem cell niche.

Since these types of events are potentially reversible, targeting such events may become clinically useful. These new observations identify novel opportunities. They provide potential markers for determining if someone is susceptible to cancer, as well as, providing potential targets for prevention and therapy. We hypothesize that these properties are critically relevant to the formation of cancer and will provide insights into the role of epigenetic modifications in disease processes and stem cell characteristics.

Role of HDAC in human stem cells pluripotentiality and differentiation

The J. David Gladstone Institutes
Principal Investigator: Eric M. Verdin, M.D. Leon J. Thal SEED Grant Program

Stem cells are able to develop into most of the specialized cells and tissues of the body and therefore have the potential to replace diseased cells with healthy functioning ones. It is the hope of the scientific and medical communities that the use of stem cell based therapies to treat diseases such as Alzheimer's disease, diabetes, heart disease and other degenerative conditions will one day be routine. Because this research field is still in its infancy, a number of scientific challenges must be overcome before promise of stem cells can be harnessed. In particular, we need to increase our understanding of the growth conditions, cellular biology and genetic events involved in stem cell survival and differentiation are key. While more than 100 distinct stem cell lines have been derived, less than 20 are available in sufficient quantities for research purposes and of these, only a very limited number have studied with respect to understanding how stem cells grow and develop into target cells. Clearly there is a great need to study more cell lines to allow comparative analysis of growth conditions, signaling and gene expression processes. These studies will help clarify how these cells can be grown to sufficient quantities to be used clinically and will also help determine at stage these cells have maximum therapeutic potential.

We are interested in understanding in molecular details two key properties of stem cells. First, self renewal is defined as the ability of stem cells to divide indefinitely, in contrast to non-stem cells which are limited in their ability to divide. Second, pluripotentiality refers to the ability of stem cells to differentiate in all cell types that are present in an adult organism. There is growing evidence that these two properties of stem cells are controlled at the central level via the interplay of cellular factors that control the transfer of DNA into RNA. Several key transcription factors have been identified that are unique to stem cells. Our laboratory has specialized during the last 20 years in the study of a family of proteins called histone deacetylases that control the activity of many transcription factors. However, no data exist on the possible role of these proteins in the self renewal and pluripotentiality of stem cells. We propose a series of experiments that will explore the role of histone deacetylases in these critical properties of stem cells.

This information will ultimately advance our efforts at generating stem cells with therapeutic potential for use in the clinic.

Self-renewal of human embryonic stem cells
University of Southern California
Principal Investigator: Qilong Ying, Ph.D. Leon J. Thal SEED Grant Program

Human embryonic stem (ES) cells are a remarkable cell type that are derived from a group of cells called the inner cell mass (ICM) of a very early stage embryo (about 100 cells in total) obtained from in vitro fertilization program. Human ES cells can be expanded in culture in an undifferentiated state (self-renewal) without limit while retaining the capacity to differentiate into nearly any type of cell. Human ES cells offer an important renewable resource for future cell replacement therapies for many diseases such as Parkinson's disease, spinal cord injury etc. However, before the full potential of human ES cells can be exploited in the clinic, we need to understand more about human ES cells so we can control their fate towards either self-renewal or towards differentiation into a specific cell type required for cell replacement therapy. Currently it is a problem just to grow human ES cells, let alone to understand how human ES cells make their choice between self-renewal and differentiation. In contrast, several signaling pathways which are important for mouse ES cell self-renewal have been identified, and as a result of this, it is possible to grow mouse ES cells in a fully defined condition. However, these pathways seem to be not operating in human ES cells. This would argue that human ES cells are very different from mouse ES cells, and that understanding of human ES cells may not benefit from the research of mouse ES cells. However, we have recently made striking discoveries on mouse ES cells. We found that for mouse ES cell self-renewal does not require any added growth factors or cytokines but only the elimination of signals that induce differentiation. These new findings provide us with a new prospective to understand human ES cells. Through understanding some of the basic mechanisms involved in human ES cell maintenance, we should be able to develop a more efficient and better method to grow human ES cells, which is clearly important if these cells are to be used clinically.

Improved hES Cell Growth and Differentiation
University of California, Irvine
Principal Investigator: Peter Donovan, Ph.D. Leon J. Thal SEED Grant Program

Human embryonic stem (hES) cells are pluripotent stem cells that can theoretically give rise to every cell type in the human body. Consequently, hES cells have enormous promise for the treatment of human disease. Specialized cell types derived from hES cells could be used to treat a wide variety of diseases and disorders including spinal cord injury, Parkinson's disease, heart disease and diabetes to name just a few. Such specilaized cells, derived from either normal hES cells or hES cells derived from embryos representative of specific disease states could also be used to screen for drugs that would ameliorate the disease. Finally, the analysis of hES cell differentiation into specialized cell types could reveal important

information about the embryonic and fetal development of our own species. This in turn could allow a better understanding of the factors that affect the growth of the human embryo and fetus and how these processes sometime go wrong leading to birth defects. But significant hurdles must be overcome if hES cell-derived cells are to be used in these ways. Growth and expansion of hES cells is still problematical. To overcome these problems we have developed methods for genetically manipulating hES cells with very high efficiency. These methods will be applied to studying the growth of hES cells. Improved methods for understanding how to grow and expand hES cells will allow expansion of hES cells in large quantities. This will be necessary in order that hES cells can then themselves be used to produce the numbers of specialized cells required either for transplantation or for drug screening. In addition, the ability to genetically manipulate hES cells will allow the mechanisms by which they can turn into specialized cells to be studied and developed in new ways. These studies should speed up efforts to make specialized cell types which can be used either to treat diseases directly or to develop drugs with which to treat those diseases. Understanding how hES cell grow should allow us to avoid one of the major problems with this technology, namely that the hES cells themselves can form tumors which may harm, rather than help, patients. Finally, hES cells are derived from the early embryo and are very similar to cells of the embryo. Therefore, understanding how hES cells grow could also inform us about the factors required for the growth of the early embryo. Consequently, these studies could have a major impact on our understanding of early embryo growth, the factors that cause certain types of infertility and, ultimately, lead to improved methods for treating infertile couples.

Mechanisms to maintain the self-renewal and genetic stability of human embryonic stem cells

University of California, San Diego
Principal Investigator: Yang Xu, Ph.D. Comprehensive Research Grant Program
Also referenced in: Genomic Instability and Cancer

Human embryonic stem cells (hESCs) are capable of unlimited self-renewal, a process to reproduce self, and retain the ability to differentiate into all cell types in the body. Therefore, hESCs hold great promise for human cell and tissue replacement therapy. Because DNA damage occurs during normal cellular proliferation and can cause DNA mutations leading to genetic instability, it is critical to elucidate the mechanisms that maintain genetic stability during self-renewal. This is the overall goal of this proposal. Based on our recent findings, I propose to investigate two major mechanisms that might be important to maintain genetic stability in hESCs. First, I propose to elucidate pathways that promote efficient DNA repair in hESCs. Second, based on our recent findings, I hypothesize that another primary mechanism to maintain genetic stability in self-renewing hESCs is to eliminate DNA-damaged hESCs by inducing their differentiation. Therefore, I propose to identify the pathways that regulate the self-renewing capability of hESCs in the presence and absence of DNA damage. In summary, the proposed research will contribute significantly to our understanding of the pathways important to maintain self-renewal and genetic stability in hESCs. This information will provide the foundation to improve the culturing condition of hESCs to promote efficient self-renewal with minimum genetic instability, a prerequisite for the development of hESCs into human therapeutics.

One major objective of the proposed research is to improve the genetic manipulation technologies in hESCs, including transgenic and gene targeting technologies. While mouse models are valuable tools to study the mechanisms of the pathogenesis in human diseases, many differences between mouse and human cells can lead to distinct phenotypes as well as the common phenomenon that certain therapeutic interventions work well in mouse models but poorly in humans. Therefore, it is of high priority to create disease-specific hESCs as powerful genetic tools to study the mechanism of the pathogenesis in human diseases. In addition, the unlimited supply of primary cells derived from the disease-specific hESCs will become valuable reagents for drug discovery. There are two ways to generate the disease-specific hESCs. One approach is through nuclear transfer that has been proven extremely difficult in human context and so far unsuccessful. The other is to employ the transgenic and gene targeting techniques to

create disease-specific hESCs. Therefore, the proposed research will significantly improve our capability to generate disease-specific hESCs. After experimenting with various existing hESC lines, we found that only the non-federally-approved hESC lines developed recently at Harvard University is most suitable for genetic manipulation technologies. Since the research involving the HUES lines can not be supported by federal government, CIRM is in a unique position to support this proposed research.

PLURIPOTENCY AND DIFFERENTIATION

The astonishing ability of embryonic stem cells to give rise to all the different tissues of the body lies at the heart of their potential to cure a wide variety of human diseases and injuries. Pluripotency is defined as the ability of stem cells to generate mature cells of many different types of tissues. Maintaining human embryonic stem cells in a state of pluripotency in culture is challenging, but essential, for the successful development of stem cell-based therapies. Another important area of research is to learn how to guide stem cells from an immature, pluripotent state to generate specific types of mature cells and tissues; this can be helped by understanding how stem cells normally differentiate and organize to form tissues. In 2007, 23 awards are approved for funding studies to investigate the mechanisms responsible for pluripotency and studies to direct the differentiation of human embryonic stem cells.

LEON J. THAL SEED GRANT PROGRAM

Profiling surface glycans and glycoprotein expression of human embryonic stem cells
University of California, Berkeley
P.I.: Carolyn Ruth Bertozzi, Ph.D.
$500,000 for 2 years

Programmed Cell Death Pathways Activated in Embryonic Stem Cells
Buck Institute for Age Research
P.I.: Dale Eric Bredesen, M.D.
$735,000 for 2 years

Combinatorial Platform for Optimizing Microenvironments to Control hESC Fate
University of California, San Diego
P.I.: Shu Chien, M.D., Ph.D.
$640,000 for 2 years

A method to maintain and propagate pluripotent human ES cells
The Salk Institute for Biological Studies
P.I.: Senyon Choe, Ph.D.
$800,000 for 2 years

Role of Glycans in Human Embryonic Stem Cell Conversion to Neural Precursor Cells
Burnham Institute for Medical Research
P.I.: Hudson H. Freeze, Ph.D.
$760,000 for 2 years

Modeling Human Embryonic Development with Human Embryonic Stem Cells
University of California, Los Angeles
P.I.: William E. Lowry, Ph.D.
$575,000 for 2 years

Regulation of human neural progenitor cell proliferation by Ryk-mediated Wnt signaling
University of Southern California
P.I.: Wange Lu, Ph.D.
$670,000 for 2 years

Production of Oocytes from Human ES Cells
University of California, Irvine
P.I.: Grant R. MacGregor, Ph.D.
$625,000 for 2 years
Also referenced in: New Stem Cell Lines and Technologies

Mitochondrial Dysfunction in Embryonic Stem Cells
University of California, Irvine
P.I.: Vincent Procaccio, M.D., Ph.D.
$635,000 for 2 years

Dollar amount shown is the amount requested rounded to the nearest $5,000, not the amount awarded.

hESCs for Articular Cartilage Regeneration
University of California, Davis
P.I.: Hari A. Reddi, Ph.D.
$370,000 for 2 years

Mapping the transcriptional regulatory elements in the genome of hESC
Ludwig Institute for Cancer Research
P.I.: Bing Ren, Ph.D.
$695,000 for 2 years

In Vitro Differentiation of T cells from Human Embryonic Stem Cells
University of California, Berkeley
P.I.: Ellen A. Robey, Ph.D.
$500,000 for 2 years

Non-coding RNA as tool for the active control of stem cell differentiation
University of California, Riverside
P.I.: Frank Uwe Sauer, D.Habil.
$600,000 for 2 years

Role of Mitochondria in Self-Renewal Versus Differentiation of Human Embryonic Stem Cells
University of California, Los Angeles
P.I.: Michael Alan Teitell, M.D., Ph.D.
$640,000 for 2 years

Analysis of Candidate Neural Crest Cells Derived from Human ES Cells
Burnham Institute for Medical Research
P.I.: Alexey Terskikh, Ph.D.
$760,000 for 2 years

Reprogramming Differentiated Human Cells to a Pluripotent State
Stanford University
P.I.: Thomas J. Wandless, Ph.D.
$650,000 for 2 years

Force, Dimensionality and Stem Cell Fate
University of California, San Francisco
P.I.: Valerie Marie Weaver, Ph.D.
$565,000 for 2 years

Role of Chromatin Modifiers in Regulating Human Embryonic Stem Cell Pluripotency
Stanford University
P.I.: Joanna Wysocka, Ph.D.
$660,000 for 2 years

Dollar amount shown is the amount requested rounded to the nearest $5,000, not the amount awarded.

COMPREHENSIVE RESEARCH GRANT PROGRAM

Regulated Expansion of Lympho-hematopoietic Stem and Progenitor Cells from Human Embryonic Stem Cells (hESC)
Childrens Hospital of Los Angeles
P.I.: Gay Miriam Crooks, M.D.
$2,555,000 for 4 years

Constructing a fate map of the human embryo
University of California, San Francisco
P.I.: Susan J. Fisher, Ph.D.
$2,535,000 for 4 years

Understanding hESC-based Hematopoiesis for Therapeutic Benefit
University of California, San Francisco
P.I.: Andrew D. Leavitt, M.D.
$2,570,000 for 4 years

Guiding the developmental program of human embryonic stem cells by isolated Wnt factors
Stanford University
P.I.: Roel Nusse, Ph.D.
$2,355,000 for 4 years

microRNA Regulation of Cardiomyocyte Differentiation from Human Embryonic Stem Cells
The J. David Gladstone Institutes
P.I.: Deepak Srivastava, M.D.
$3,165,000 for 4 years

Dollar amount shown is the amount requested rounded to the nearest $5,000, not the amount awarded.

ABSTRACTS

[Provided by applicant]

Profiling surface glycans and glycoprotein expression of human embryonic stem cells
University of California, Berkeley
Principal Investigator: Carolyn Ruth Bertozzi, Ph.D. Leon J. Thal SEED Grant Program

Human embryonic stem cells can be changed into virtually any cell type in the adult body. Because of this unique capability, these cells have the potential to cure a vast majority of existing human disorders. Several hurdles exist, however, which need to be overcome before results from the exciting field of stem cell research can be used in the clinic. For example, the factors which govern conversion of stem cells into a variety of tissue types that may find uses in regenerative medicine such as in liver, heart, brain, are not well understood. Our research employs a unique multidisciplinary and collaborative approach to harness the expertise of several leading scientific laboratories to bridge this information gap. In particular, our area of specialization is in understanding how the sugars which coat the surfaces of cells impact processes such as the malignant transformation of cancer cells. The CIRM grant will enable us to apply this same accumulated expertise to study the roles of cell surface sugars in the transformation of human embryonic stem cells into cell types useful for the treatment of human diseases.

Programmed Cell Death Pathways Activated in Embryonic Stem Cells
Buck Institute for Age Research
Principal Investigator: Dale Eric Bredesen, M.D. Leon J. Thal SEED Grant Program

The therapeutic potential of human embryonic stem cells is extraordinary. Without a doubt, regenerative medicines will save thousands of lives in the years to come. Before that day arrives, much needs to be learned from the cells themselves. The reasons that these cells hold so much promise are two-fold: (1) embryonic stem cells can renew themselves indefinitely (divide and divide and...) and (2) embryonic stem cells can be trained to become any cell type of the body (neurons, heart muscle, skin, liver, kidney...). However, it should be emphasized that these two points are only valid if the growth conditions are properly established. While we have made great strides in developing culture conditions that can support self-renewal of embryonic stem cells, we are a long way from mastering the conditions necessary for differentiating embryonic stem cells into every cell type of the body (of which there are about 200). Ultimately, if therapies based on stem cells are to be realized, these cells will have to be grown in massive quantities, with an unprecedented level of quality control to ensure that only one cell type can be found in the lot. Furthermore, the fate of stem cells is crucial to their use in new therapies in other words, these cells must be kept alive and functional to have benefit to human patients.

However, one of the major challenges facing the growth of embryonic stem cells is the abundance of cell death that occurs. Cells typically die when their needs are not met (either lack of proper nutrients or growth factors) or when they face harsh conditions. If we could somehow block the cell death that occurs in these cultures or if we could change the conditions to remove the components that trigger cell death, we could achieve growth of hESCs of a greater scale. It turns out that when cells die, they do not do so passively. Instead, once given a "go" signal, cells utilize their own energy and cellular machinery to dismantle themselves, a process known as programmed cell death. There are at least five major forms of programmed cell death: apoptosis (the best described pathway), autophagic cell death, PARP-mediated cell death, paraptosis, and calcium-mediated programmed cell death. Each of these programmed cell death pathways are activated by different stresses. In the proposed research, we aim to determine which of the five major forms of programmed cell death occur in hESCs. Furthermore, we will evaluate how the repertoire of PCD pathways changes when hESCs change, or differentiate, into neurons. At the same time that we will be learning about the most appropriate conditions for growing hESCs, we will also be

able to determine which conditions are ideal for cultivating neurons, which could ultimately be used in regenerative medicine therapies.

Combinatorial Platform for Optimizing Microenvironments to Control hESC Fate
University of California, San Diego
Principal Investigator: Shu Chien, M.D., Ph.D. Leon J. Thal SEED Grant Program

The aim of California Stem Cells Initiative is to develop new therapeutical approaches by utilizing human embryonic stem cells (hESCs) to renew themselves and to differentiate into a variety of cell types, thus enabling the engineering of specific tissues to treat diseases that cannot be currently cured. To realize the potential of hESCs in regenerative medicine will require (1) the establishment of conditions for the expansion of these cells into a sufficiently large quantity and (2) the development of protocols to differentiate them into specific cell types and generate the desired tissues. Experimental manipulation of the environmental cues, such as chemical signals and physical stresses, to which stem cells are exposed, will lead to the discovery of conditions that specifically direct hESC growth and differentiation.

Studies on factors affecting stem cell growth and differentiation tend to focus on one or a few elements in the microenvironment, e.g., some proteins in the matrix underlying the cell or growth factors brought to the cell from the circulation or neighboring cells. The proposed research will develop a platform that will allow the concomitant screening of thousands of conditions consisting of combinations of various molecules for the systematic selection of the optimum conditions for hESC growth and differentiation. This platform is based on a microarray technology using robots to place 1200 spots of individual and combinations of proteins in a precise pattern on a glass slide surface. The hESCs attached to these spots will interact with different molecules in the patterned spots to elicit specific cellular responses. In addition, we will subject the cells on the arrays to well-controlled mechanical forces imposed by fluid shearing. Thus, we will combine mechanical and molecular stimuli in a controlled manner to study the responses of hESCs to physicochemical modulations in their microenvironment in terms of their signaling behavior and cellular fate, i.e., growth and differentiation.

The application of hESCs for regenerative medicine requires the establishment of the optimal physicochemical microenvironment that allows us to control and direct the growth and differentiation of these cells. Our proposed research focuses specifically at this critical need. We will develop a "systems" approach to understanding the response of hESCs to multiple factors in the microenvironment. The results will lead to the definition of the optimal microenvironment parameters for the control of differentiation of hESCs into specific cell types such as cardiovascular cells, neuron cells, cartilage/bone cells, etc., for the treatment of many important human diseases. Hence, this project has fundamental importance and broad applications. This is a most cost-effective way to pursue hESC research for the improvement of human health and quality of life, and the resulting technology advances may provide financial gain for the people in California.

A method to maintain and propagate pluripotent human ES cells
The Salk Institute for Biological Studies
Principal Investigator: Senyon Choe, Ph.D. Leon J. Thal SEED Grant Program

Human embryonic stem (hES) cells are pluripotent such that they can differentiate into all three germ layers, thus potentially all different types of tissues of the body. Pluripotency is characteristic of only embryonic cells, but it can also be achieved by reprogramming differentiated cells by transferring nuclear contents into unfertilized, enucleated oocytes or by fusing with ES cells. To achieve the initial embryo-like state, it is a pre-requisite to be able to maintain and propagate these ES cells in culture conditions in vitro. Currently, such recipe exists for mouse ES cells. Surprisingly, similar media components for hES cells do

not work. This very first technical barrier needs to be overcome in order to realize full clinical potential of stem cell therapy.

We propose to develop a novel recipe of chemically defined culture media and culture conditions to grow and maintain pluripotency of hES cells. The media we will evaluate are combinatorial mixtures containing only recombinant proteins, chemically synthesizable reagents, or human source factors. To achieve new sets of recombinant protein reagents known to be involved in controlling differentiation and pluripotency of embryo-like cells, we will develop a novel biochemical strategy of producing a set of target protein reagents effectively in test tubes. To screen conditions using these chemically defined components and various culture conditions, we will develop a new cell line containing a reporter gene (GFP) recombined into human Oct4 gene. Human Oct4 gene is the prominent marker for stemness of the hES cells.

There are three specific Aims for this proposed study. They are, 1) production of the media components biochemically, 2) development of two Oct4-reporting hES cell lines, and 3) screening of culture media and conditions for maintaining pluripotency of hES cells. These experiments will be carried out in parallel as collaboration between two laboratories Senyon Choe and Juan-Carlos Belmonte. Once Aims 1 and 2 are completed, we will evaluate these hES cell lines in various culture conditions systematically (Aim 3). In doing these high-throughput assays for functional characterization, we will also conduct screening of known chemical library of selected drugs and metabolites to glean into their potential ability to augment or inhibit actions of the engineered biologic reagents in controlling the growth and pluripotency of hES cells. From the screening using these two cell lines, we will establish the firm method of propagating and maintaining pluripotency of hES cells for subsequent clinical applications.

Role of Glycans in Human Embryonic Stem Cell Conversion to Neural Precursor Cells
Burnham Institute for Medical Research
Principal Investigator: Hudson H. Freeze, Ph.D. Leon J. Thal SEED Grant Program

Like a thick frosting on a cake, complex sugar chains decorate every surface of every cell. Try to approach a cell, as friend or foe, and the canopy of sugars is the first gate-keeper. Each cell makes and organizes these sugar chains, called glycans, on its surface. They are very complicated molecules, and different cells choose to decorate themselves with different glycans-- for reasons best known to the cells themselves. Because glycans have such complicated structures, it is hard to work with them and understand their function. They are much more diverse than DNA and proteins, and so the technology for dissecting their structures and functions has lagged behind the others in the molecular revolution in biology and medicine. Glycans are complicated molecules, hard to work with, difficult to understand, but they are absolutely indispensable to life.

Human genetic disorders where just one step in their assembly is missing causes mental retardation, seizures, blindness and poor motor skills.

Glycans are used for communication both within and between cells, and this is especially true when cells signal each other about their past and future journeys within the developing body and exactly where they will go and what they will become during development.

Embryonic stem cells have a particular set of glycans on their surface and change as the cells develop into different cell types. What directs these changes? Are they all important? Can we manipulate a cell's fate or convince it to behave in a certain way by changing-”or maintaining'” the sugar coating? The scientific literature shows that changing surface sugar chains can have profound effects.

New technology in the field of "Glycobiology" makes it possible to analyze minute amounts of material with great precision and define these structures. Thanks to our collaborators, we can produce substantial amounts of human embryonic stem cells that uniformly transition into neural precursor cells. Our plan is to describe in detail these glycan changes as they occur and then determine which are actually essential for

cells to reach that point. How do these glycans allow them to go further on to neurons, oligodendrocytes and astrocytes? We hope to exploit these unique sugar signatures to identify and isolate cells that will have a particular developmental fate. This is only the beginning. It is a catalog of events and a parts list, but we know how the parts are assembled and what machines are needed. Since we are only beginning the stem cell enterprise, it's important to define these elements from the beginning. We hope to use this knowledge to direct and influence stem cells to travel down the paths we prefer, since we already know the path is sugar coated and the coating is essential.

Modeling Human Embryonic Development with Human Embryonic Stem Cells
University of California, Los Angeles
Principal Investigator: William E. Lowry, Ph.D. Leon J. Thal SEED Grant Program

Stem cells have entered the public consciousness as "cells that can do anything" and have been hailed as a panacea in the fight against disease, aging and cancer. Unfortunately, we have only scratched the surface in understanding these cells. Some of the things we think we know are that: embryonic stem cells hold great promise because they do seem to be "cells that can do anything", but still cannot be isolated from consenting adults, and that adult stem cells, while isolatable, are much more limited in their ability to replenish tissue beyond their organ of origin. In addition, we know very little about human embryonic development for the simple fact that experiments on human embryos has proven to be nearly impossible due to ethical and technical obstacles. Clearly, if we gained a deep understanding about human embryos and human embryonic stem cells, we could not only develop useful clinical opportunities, but also potentially detect and treat errors made during human development. This proposal suggests that in fact we could learn a great deal about not only the therapeutic potential of hESCs, but also human development by exploiting cell culture. We propose to model human embryonic development in order to understand how a particular portion of the embryo undergoes a transformation to become either the brain or the skin. The fact that seemingly one cell type early on in the embryo can form either the complete nervous system or the skin has intrigued scientists for decades, we now hope to understand how this process works and in the process we hope to challenge existing theories of the potential of adult stem cells as well. With a deeper understanding of what makes a neuron a neuron as opposed to a skin cell, we will in fact be able to impart a neural code on a skin cell, and perhaps turn a skin cell into a neuron. If this becomes possible, we could: take a skin biopsy from a patient with parkinson's disease (a degenerative disorder where dopaminergic neurons are lost), use already established mechanisms for expanding those skin cells in culture, turn on the 'neural code' to turn them into dopaminergic neurons, and then transplant them back into the same donor patient. This kind of self-transplant obviates the need for either immuno-suppression therapy which is toxic and sometimes deadly, or for patient-specific stem cells which are, for now, impossible to derive.

Regulation of human neural progenitor cell proliferation by Ryk-mediated Wnt signaling
University of Southern California
Principal Investigator: Wange Lu, Ph.D. Leon J. Thal SEED Grant Program

Human ES cells can be used to make healthy neurons to replace the cells that are lost in neurological diseases such as Alzheimer's and Parkinson's diseases. This holds great hope for future treatment of these disorders. Our research aims to produce neurons from ES cells more efficiently. During the process of differentiation to form nerve cells, human ES cells first develop into primitive neural progenitor cells before they become mature functional neurons. Our research focuses on how we can expand the neural progenitor cell population.

We have found that a cell surface protein called Wnt can expand neural progenitor cells. However, it is not clear how this protein can make human neural progenitor cells grow. This proposal will address the question. We will determine which surface molecules can recognize Wnt and how these signals control

cell growth. This proposed research will help us to find a method to expand neural progenitor cells and thus make more neurons in the future. This will eventually contribute to the clinical application of human embryonic stem cells in the treatment of neurological diseases.

Production of Oocytes from Human ES Cells
University of California, Irvine
Principal Investigator: Grant R. MacGregor, Ph.D. Leon J. Thal SEED Grant Program
Also referenced in: New Stem Cell Lines and Technologies

The ability of human embryonic stem (hES) cells to form a wide variety of adult human cell types offers hope for development of novel therapies to treat human degenerative diseases such as Alzheimer's, diabetes, and muscular dystrophy. However, to prevent rejection of the transplanted cells by a patient's immune system it will be important to use hES cell derived tissues that are immunologically matched to the patient. One way to do this involves somatic cell nuclear transplantation (SCNT) where the nucleus containing the genetic information is transferred from a patient's cell into a human oocyte (egg) from which the nucleus has been removed. The oocyte is then stimulated to divide into a small group of cells from which new hES cells are derived. As cells derived from these hES cells contain the patient's DNA they will be immune-matched to the patient, thereby preventing tissue-rejection.

While SCNT has been performed using cells and eggs from mice, it is not yet possible to do this on a routine basis using human cells and eggs. One reason for lack of progress concerns the scarce supply of human oocytes available for research. Oocytes are usually obtained from women undergoing hormonal treatment for infertility, when permission is given for unused and unwanted oocytes to be used for research. However, a severe shortage and great demand for such material has stimulated efforts to recruit suitable donors from the general public, often with financial incentive. There is significant health and ethical concern about such policies and the potential negative impacts of such procedures on long-term health of women are unclear.

Remarkably, recent research with mice suggests that it may be possible to produce oocytes from hES cells. If so, the oocytes produced may be of use for SCNT to produce new lines of personalized-hES cells for treatment of patients. If successful, this would be expected to have at least two major benefits to the public. First, women would no longer be required as a source of eggs for research, which would reduce the risk of such treatments on women's long-term health. Second, this would generate a theoretically infinite increase in the quantity of oocytes available for research, which would in increase the rate at which technical advances could be made in production of immune-matched hES via SCNT.

Consequently, the proposed research will investigate methods for production of germ cells and oocytes from female hES cells. Specifically, we will test our prediction that it is possible to coax hES cells in culture to form germ cells and ultimately mature oocytes by exposing hES cells to different cell proteins and hormones that are normally used by the body to generate eggs. If successful, these protocols will help accelerate research on development of therapeutic cloning for a wide range of diseases. This would also obviate ethical concerns regarding egg-donation by women and would help protect women's health.

Mitochondrial Dysfunction in Embryonic Stem Cells
University of California, Irvine
Principal Investigator: Vincent Procaccio, M.D., Ph.D. Leon J. Thal SEED Grant Program

A major concern for the utilization of human Embryonic Stem Cells (hESCs) for cell replacement therapy is that with prolonged culture, the capacity of the cells to generate the desired cell types for therapy declines. While the reason for this is currently unknown, our research suggests that an important factor is

damage to the genetic blueprints that are necessary to sustain the cellular power plants of the cell, the mitochondria.

The human cell is the product of a symbiotic merger that occurred two billion years ago of two different cell types: one generating the host cell and the other generating an intra-cellular colony of bacteria, the mitochondria. In the modern human cell, the host cell constitutes the nucleus and the cytosol and the genetic information (DNA) for this nucleus-cytosol organism resides in the nucleus and is responsible to building and maintaining the structural elements of the cell: analogous to the carpenters blueprints for building a house. The mitochondria have their own DNA blueprints, the mitochondrial DNA (mtDNA), and this describes the circuit diagram for the energy production system of the mitochondria: analogous to the electrician's wiring diagram for the house.

Mutations which damage the mtDNA circuit diagram result in the mitochondria's inability to repair damage to the mitochondrial energy production system. As the efficiency of the mitochondrial power plants declines, they make less energy and more smoke, the smoke generated being oxygen radicals. As oxygen radical production increases, it causes increased damage to the mitochondria and mtDNAs, ultimately resulting in the mitochondrial power plants go off-line and the death of the cell.

As mitochondrial oxygen radical production increases, it stimulates the cell to divide in an effort to dilute out the smoke generating mitochondria. However, the problem is that the damaged mtDNA blueprints replicate along with the cell. Hence, the toxicity continues to increase.

We and others have documented this type of phenomenon occurs in a variety of cultured cell types. Therefore, it is likely that it also occurs in hESCs. If so, as damage to the mtDNA accumulates, hESC energy production declines and oxygen radical production increases until the hESC is no longer capable of building the more complex structures necessary to create tissue replacement cells.

If we can prove that this scenario does occur in hESCs, then we can develop drugs that will limit mitochondrial oxygen radical production and protect the mitochondria and mtDNAs from oxygen radical damage. Furthermore, we have developed a method that permits us to replace damaged mtDNAs in cells with new one. Hence we could repair the mtDNA damage of aging hESCs and regenerate their capacity to make high quality differentiated cells for use in tissue replacement therapy.

hESCs for Articular Cartilage Regeneration
University of California, Davis
Principal Investigator: Hari A. Reddi, Ph.D. Leon J. Thal SEED Grant Program

Mobility is critical for human social interactions and quality of life. In the aged mobility is progressively impaired due to painful joints. The articular cartilage in the joints is damaged. The long-term goal of our research is the utilization of human embryonic stem cells (hESCs) for cartilage formation and therefore, regeneration of articular cartilage. Stem cells have enormous potential. Their potential can be directed by morphogens governing chondrogenesis. Bone and cartilage morphogenetic proteins induce stem cells to form cartilage cells. This research will contribute directly to the development of therapy for osteoarthritis for the aging Californians.

Mapping the transcriptional regulatory elements in the genome of hESC
Ludwig Institute for Cancer Research
Principal Investigator: Bing Ren, Ph.D. Leon J. Thal SEED Grant Program

The human embryonic stem cells (hESC) have the remarkable potential to replicate themselves indefinitely and differentiate into virtually any cell type under appropriate environmental conditions. They

accomplish this through regulating the production of a unique set of proteins in the cells, a process known as gene regulation. While the genes encoding these stem cell proteins have been largely identified over the years, the mechanisms of gene regulation are not yet understood. This gap in our knowledge has seriously limited our ability to manipulate hESC for therapeutic purposes.

In Eukaryotic cells, gene regulation depends on specific sequences in the DNA known as transcriptional regulatory elements. These regulatory DNA consists of promoters, enhancers, insulators and other regulatory sequences. As a key step towards understanding the gene regulatory mechanisms in hESC, we will produce a comprehensive map of promoters, enhancers and insulators in the hESC genome. We will use a newly developed, high throughput experimental strategy to identify these sequences that are engaged in gene activation in hESC. Our strategy involves identifying the DNA sequences that are associated with the specific transcription factors or chromatin modification signatures known to be present at each type of regulatory elements inside the hESC. We will use biochemical procedures to isolate these sequences from the cell and determine the resulting DNA in large scale with the use of DNA microarrays, containing of millions of DNA species that together represent the complete genomic makeup of the hESC. Completion of the proposed research is expected to improve our knowledge of the gene regulatory mechanisms in hESC, which in turn will facilitate the development of new strategies for stem cell based therapeutics.

In Vitro Differentiation of T cells from Human Embryonic Stem Cells

University of California, Berkeley
Principal Investigator: Ellen A. Robey, Ph.D. Leon J. Thal SEED Grant Program

White blood cells are the main players of human immunity in defense against infection. Defects in CD4 T white blood cells, for example, can lead to the devastating infections observed in AIDS patients and patients with a genetic immunodeficiency syndrome ("bubble boy" syndrome). A normal immune system can recognize and attack pathogens but not "self". This is achieved by rigorous selection and "education" during development of T or B white blood cells and by regulatory T cells that suppress occasional "run-away" white blood cells. Glitches in these processes can also lead to equally devastating problems as seen in many autoimmune diseases like type I diabetes, multiple sclerosis and rheumatoid arthritis. Thus, the availability of T cells or regulatory cells could lead to therapies for many human diseases.

A major limitation in using T cells in therapy is the lack of available primary T cells generated in the laboratory. Attempts thus far to generate T cell precursors in a tissue culture dish from the existing NIH approved embryonic stem (ES) cell lines have had only limited success, perhaps due to the partial white blood cell potential of these particular lines. Efficient conversion of T cell precursors into mature T cells or regulatory T cells in a tissue culture dish have also not yet been achieved.

In this seed grant application, we propose to test a series of new (non-federally approved) human embryonic stem (ES) cells for their abilities to efficiently generate white blood cells precursors and subsequently immature T cells in a tissue culture dish. Conditions to optimize generation of white blood cell precursors and T cell precursors will be sought. We will then examine how to convert mature T cells from T cell precursors by varying the culture conditions to closely mimick the "real" situation in humans. Successful completion of this project would constitute a major first step in the long-term goal of using human T cells (that can attack pathogens, but do not react to one's own organs) in therapy. Examples of possible clinical therapy include administration of regulatory T cells in preventing autoimmune attacks in a variety of aforementioned autoimmune diseases. Availability of T cells specific for proteins found in tumors could be used to treat cancer, and mature T cell populations could be used to restore immune function of AIDS patients and to improve the short-term survival of bone marrow transplant recipients.

Non-coding RNA as tool for the active control of stem cell differentiation
University of California, Riverside
Principal Investigator: Frank Uwe Sauer, D.Habil. Leon J. Thal SEED Grant Program

Stem cells are multipotent, meaning that they can develop into any cell type of the human body. Biomedical applications propose that, after introduction into humans, stem cells could replenish damaged or lost cells in human bodies and thereby cure human diseases such as Parkinson, Alzheimer's, and diabetes. One prerequisite for the success of the biomedical application of stem cells are tools that actively control the development of a stem cell into any given cell type, such as neurons, muscle cells, or insulin-producing pancreatic cells. However, the tools directing stem cell differentiation are only now being discovered. The proposed research project intends to fill this gap and uses a novel research approach to develop tools, which can control to development of cells (cell differentiation) into any desired cell type.

The research approach is based on studies, indicating that a novel group of non-coding RNAs plays an important role in cell differentiation in the fruit fly and mice. The non-coding RNA originate from and control the expression of genes, whose activities control cell differentiation. We have shown that the introduction of non-coding RNA into cells changes the developmental fate of cells, suggesting that the non-coding RNA represent tools that control the differentiation of cells including stem cells. We have identified 32 non-coding RNAs in human cells, which originate from different regulators of cell differentiation and are transcribed in differentiated but not human stem cells. Thus, the specific working hypothesis of the research project is that non-coding RNA control the differentiation of human stem cells.

To test that hypothesis, we shall assess whether non-coding RNA can induce stem cell differentiation. First (Aim 1), we shall test whether the introduction of non-coding RNA into human stem cells activates the expression of genes, whose activities control cell differentiation. To confirm the results of Aim 1, we shall assess whether non-coding RNA-mediated expression of key regulatory genes of cell differentiation coincides with the recruitment of regulatory proteins, which establish and maintain the expression of the key regulatory genes throughout the entire life. This is important, as an actively controlled progression of stem cell to differentiated cell is only then successful, when the differentiated cell maintains its identity throughout the entire life. Third, we shall elucidate whether activation of key regulatory genes by non-coding RNA induces cell differentiation. In summary, the proposed project will provide novel insights into the molecular mechanisms underlying stem cell differentiation and novel molecular tools to control stem cell differentiation. Our efforts will significantly contribute towards the development of biomedical applications that allow the utilization of stem cells in the treatment of human diseases.

Role of Mitochondria in Self-Renewal Versus Differentiation of Human Embryonic Stem Cells
University of California, Los Angeles
Principal Investigator: Michael Alan Teitell, M.D., Ph.D. Leon J. Thal SEED Grant Program

Human embryonic stem cells (hESCs) hold great potential for treating multiple human dread diseases, including but not limited to cancer, diabetes, obesity, Alzheimer disease, and certain types of heart failure. However, a growing appreciation exists for the notion that not all hESCs have identical capabilities in correcting or ameliorating disease and not all hESCs will be valuable as potential therapeutic cell sources. Because hESCs contain genetic information like all human cells, some hESCs will have genetic mutations or alterations that will make them more or less desirable for therapy. The heritable information contained with hESCs comes from DNA in the cell nucleus and also from DNA within maternally inherited mitochondria. In fact, it is the functional capabilities of mitochondria in hESCs that this proposal addresses because over 400 mutations in mitochondrial DNA result in disease and many more disorders

associated with mitochondrial dysfunction, often unidentified at the molecular level, arise from mutations in nuclear DNA.

It is potentially dangerous that so little is known about the functional capabilities and role for mitochondria in hESCs and in the major decisions that hESCs make, such as whether to self-renew and make more stem cells or to differentiate into any one of the known human lineages, including muscle, skin, brain, and other cell types. We anticipate the day when stem cell therapies to combat disease or provide replacements for worn out components will be a main part of individualized medical treatment. We believe it is therefore critical to choose the best stem cell starting materials for such therapeutic applications. This view, combined with a desire to understand how mitochondria, as the main source for a cell's energy and building block generation, functions in stem cell decisions, propels us to provide 3 integrated areas of specific investigation into stem cell mitochondria and their role(s) in decision making. Our studies will evaluate basic mitochondrial functions and structures in a variety of hESCs to gain an appreciation for variability in distinct hESCs (Aim 1). We will alter mitochondrial function in hESCs with genetic and environmental insults to gain an appreciation for the global effects on hESC function and as a way to help select appropriate stem cells for future therapeutic applications (Aim 2). We will force hESCs with normal or altered mitochondria to differentiate into germ cells, blood cells, skin cells, or brain cells with expert collaborators in each lineage type to evaluate how the state of mitochondrial function will dictate the ability for hESCs to provide multiple, distinct replacement lineages for use (Aim 3).

In sum, we expect our studies will reveal the critical role for mitochondria In stem cell biology and this new knowledge and our analytical approach will help provide essential information for choosing optimum stem cells for future therapeutic applications.

Analysis of Candidate Neural Crest Cells Derived from Human ES Cells

Burnham Institute for Medical Research

Principal Investigator: Alexey Terskikh, Ph.D. Leon J. Thal SEED Grant Program

Little is known about human Neural Crest (NC) cells, a transient population of cells briefly present during very early human development; the reason why these cells are extremely difficult to obtain and study. In the model organism NC cells generate an amazing array of tissues, including peripheral and enteric nervous systems, cranial bones and cartilage, some cardiac muscle and virtually all pigmented cells in the body. Abnormalities in NC cells involved in numerous human pathologies including various skeletal syndromes (e.g. Apert syndrome), diseases of nervous system (e.g. Hirschsprung's disease) and pigment disorders (e.g. Waardenburg syndrome).The lack of fundamental knowledge about human NC impedes technological advancements. Human NC cells have never been isolated and characterized on cellular and molecular levels. The goal of this proposal is to fill this gap in our knowledge.

The PI laboratory has recently developed an efficient procedure for the rapid differentiation of human ES cells into uniform neural precursors (hES-NPCs), which was hitherto unachievable. In culture, hES-NPCs become functional neurons and oligodendrocytes. Intriguingly, our preliminary data show that many markers associated with NC cells are upregulated in hES-NPCs or during their derivation process. For instance, genes previously implicated in NC specification and maintenance were clearly detected. Moreover, a subset of hES-NPCs stained positive for cell surface antigen transiently associated with migrating NC cells in chick.

Our hypothesis is that human NC cells are present in hES-NPCs, established in our laboratory. To prove (or disprove) this hypothesis we will rigorously examine the molecular and cellular fates of candidate NC cells from hES-NPCs cultures both in vitro and in vivo.

To pursue Aim1 we will use in situ hybridization, immunostaining, prospective isolation of candidate NC cells by FACS and in vitro differentiation analysis to determine the vitro fates of candidate human NC

cells. However, the in vitro differentiation conditions for all potential human NC cell fates are unknown. In Aim2 we will determine the in vivo fates of candidate human NC cells by transplanting genetically labeled hES-NPCs and their subpopulations into the early chick embryo and following their fates during chick development. To assure the expertise and skills in the NC field (new for this PI) we have established collaboration with Dr. Bronner-Fraser (Caltech) the world's-leading expert in the NC filed.

Our analysis will unequivocally determine the in vitro and in vivo fates of hES cell-derived cells with the neural crest cell markers present in our cultures. If our hypothesis is correct, hES cell-derived neural precursors will be a rich source for neural crest cells, thus allowing for the first time the extensive characterization of these rare human cells and the development of strategies of NC cell-based protocols in clinics.

Reprogramming Differentiated Human Cells to a Pluripotent State
Stanford University
Principal Investigator: Thomas J. Wandless, Ph.D. Leon J. Thal SEED Grant Program

If the therapeutic potential of human embryonic stem (ES) cells is to be realized, the ability to produce pluripotent stem cells with defined genetic backgrounds is essential. Pluripotent cells, through differentiation, have the ability to become any cell type. For basic and applied research, access to human ES cells derived from patients with specific diseases would be very valuable. In a more therapeutic setting, the ability to isolate differentiated cells from an individual patient and reprogram these cells to a pluripotent, stem-like state may ultimately lead to truly personalized medicine. Thus, an understanding of the genes that establish and maintain the pluripotent state of human ES cells is critical to future medical applications.

The overall goal of this research program is to establish an experimental protocol to efficiently reprogram differentiated human cells into a pluripotent state. It has recently been shown that the expression of only four genes in mouse fibroblasts reprograms these cells to a pluripotent state. We will pursue a similar strategy using differentiated human cells. Importantly, we have recently developed a new technology for regulating protein expression in human cells, and this technology will allow us to regulate the expression levels of these reprogramming proteins with unprecedented control.

The first half of this proposal focuses on the regulated expression of several genes that are known to be involved in the establishment and maintenance of pluripotency in human ES cells. By using our technology to regulate the levels of these proteins in differentiated cells, we will define the expression levels that lead to efficient nuclear reprogramming. The second half of this proposal will focus on epigenetic reprogramming. Epigenetic marks are modifications to DNA and the supporting histones that do not change the actual DNA sequence but that regulate gene expression. We will use our new technology to regulate the expression of proteins that are involved in maintaining the epigenetic state that is characteristic of embryonic stem cells. We believe that expression of these epigenetic modifiers, coupled with the regulated expression of pluripotency-inducing genes, will dramatically improve the efficiency for reprogramming differentiated cells to a pluripotent state.

Force, Dimensionality and Stem Cell Fate
University of California, San Francisco
Principal Investigator: Valerie Marie Weaver, Ph.D. Leon J. Thal SEED Grant Program

Human embryonic stem cells (hESCs) are cells derived from human embryos early in development before their fate has been sealed. These cells grow and differentiate in response to a variety of stimuli to eventually give rise to all of the differentiated tissues in the body. By exploiting the remarkable potential of hESCs to differentiate into multiple cell lineages, medicine stands to benefit enormously. To do so

requires a comprehensive understanding of the optimal conditions to grow and differentiate these cells. What is known is that the physical environment in which hESCs reside plays an important role in regulating their tissue-specific differentiation.

Recent work has highlighted the importance of the composition and structure of the extracellular matrix (ECM), within which hESCs exist in vivo, in directing hESC differentiation during embryonic development. In an embryo, hESCs differentiate in a dynamic and structurally distinct three-dimensional (3D) ECM, rich in nutrients and exogenous stimuli (force). Mechanical stimulation (via matrix compliance and externally applied force) dramatically influences the formation and development of the embryo. Despite these compelling observations, information regarding the mechanisms whereby matrix compliance and external force regulate hESC differentiation in 3D is extremely limited. Instead, the majority of the research on hESCs has been in two-dimensional (2D) culture on stiff plastic substrates, despite the lack of physiological relevancy.

To address this issue, we will investigate the role of the ECM in 2D and 3D on hESC behavior using biomaterials with well-defined compositional and physical properties. We will assess the role of exogenous force by building a bioreactor designed to impart oscillatory compressive loading on hESCs cultured in 3D ECMs. We will test whether force modulates hESC fate by altering the function of the small RhoGTPase Rac. We will achieve this goal by: determining whether matrix compliance influences hESC differentiation in 2D and 3D and exploring the role of force on Rac activity and function, building a bioreactor capable of imparting controlled cyclic compressive loading to 3D hESC embedded in engineered biomaterial constructs, and by characterizing the effects of dynamic compression on hESC fate by manipulating the loading system. Because our appreciation and understanding of the mechanisms whereby matrix compliance and external force regulate hESC fate is extremely limited, this work would not likely be federally funded. These studies are essential to illustrate the critical role of matrix force in hESC fate and lay the foundation for future studies aimed at clarifying molecular mechanisms. The work will also assist in establishing defined, in vitro systems that more closely recapitulate the in vivo behavior of hESCs to permit their pluripotent propagation, and ensure their correct specification thereby ensuring the safe application of hESCs for human therapy.

Role of Chromatin Modifiers in Regulating Human Embryonic Stem Cell Pluripotency
Stanford University
Principal Investigator: Joanna Wysocka, Ph.D. Leon J. Thal SEED Grant Program

The life of every human starts with a fertilized egg. This single cell starts to divide and, in a truly amazing process, gives rise to a developed human being. Although each cell of a developed organism is a progeny of this single zygote, and shares the same genetic information with every other cell, cells differentiate to specialized forms such as skin, muscle or nervous cells. Thus, new information emerges during development, and is inherited in a fashion that does not involve changes in DNA sequence. This fascinating process is called epigenesis. Epigenetic changes underlie not only normal, but also pathological development. Abnormal epigenesis contributes to human pathology, such as aging, cancer, degenerative diseases, developmental defects and mental retardation.

Embryonic stem cells (ESCs) share with the early embryo the potential to produce every type of cell in the human body. This rare biological property is known as pluripotency. Pluripotency is a unique epigenetic state, in that ESCs can self-renew, while retaining the potential for multilineage differentiation. The research proposed here aims at elucidation of the precise molecular nature of pluripotency.

In the last decade evidence emerged that a substantial portion of epigenetic information is transmitted in a form of chemical modifications of histones and DNA, in particular histone methylation. The physiological template of our genome, called chromatin, is composed of DNA wrapped around histone proteins. Methylation marks are written and erased from histones by specific enzymatic activities and they are read

by the specialized proteins to activate or silence gene expression. Here we propose to elucidate which writers, readers and erasers of histone methylation are required for maintenance of the unique epigenetic state of pluripotency. Building on this initial knowledge we will perform a series of biochemical experiments to understand the network of protein-protein and protein-DNA interactions involved in the epigenetic regulation of pluripotency.

We are hoping that our studies will significantly advance our understanding of the unique properties of ESCs and bring us closer to the development of efficient technologies to direct the differentiation of stem cells into therapeutically useful tissues. Even more exciting is possibility that uncovered epigenetic regulators of pluripotency could be used to reset a patient's differentiated cells to the pluripotent state, thus removing the current bottlenecks in stem cell derivation and requirement for human oocytes, and sidestepping the problems of tissue rejection. Last, but not least, understanding the mechanisms of epigenetic plasticity of human ESCs will contribute to the basic knowledge of human development. Basic knowledge has proven itself time and again to be the raw fabric of innovation and progress in medicine. Thus, in the long run our research may help the humankind in ways we are not yet able to predict.

Regulated Expansion of Lympho-hematopoietic Stem and Progenitor Cells from Human Embryonic Stem Cells (hESC)

Childrens Hospital of Los Angeles
Principal Investigator: Gay Miriam Crooks, M.D. Comprehensive Research Grant Program

The clinical potential of human embryonic stem cells (hESC) for transplantation will be realized only when we can develop methods to control the process of tissue differentiation far more efficiently than is currently the case. From over 40 years of experience with adult stem cells, it is recognized that the growth of transplanted bone marrow is generated from the hematopoietic ('blood-forming') stem and progenitor cells present in the graft. Mature, differentiated cells that accompany the stem cells disappear rapidly after transplantation as they lack the ability to self renew. It is thus essential when designing clinical approaches that use tissue derived from hESC, to specifically target the production of stem and progenitors that will survive, proliferate and differentiate after transplantation. This proposal addresses three fundamental questions for the entire hESC field 1.Do hESC differentiate through the same pathways that exist in adult tissues, 2.How do the conditions in which hESC are initially derived from blastocysts affect their subsequent potential for generating tissue specific stem and progenitor cells, and 3.How can hESC differentiation be regulated to provide large numbers of tissue specific stem and progenitor cells able to engraft and differentiate long term? Studies of hematopoiesis in mice have provided the conceptual basis for the entire field of stem cell biology. However, fundamental biological and technical differences exist in both hematopoietic and embryonic stem cell biology between the murine and human species. Our group has chosen over the past 15 years to focus on the study of human hematopoietic stem cells and lymphoid (immune system) progenitors, more recently bringing these concepts and tools to study hematopoietic differentiation from hESC. In brief, our aims in this proposal are: 1. To understand the pathways along which the blood and immune system are generated from hESC, 2. To assess if the methods by which hESC are derived affect their capacity for hematopoiesis, and 3. To develop the means to expand hematopoietic stem cells derived from hESC. I believe that there are two broad reasons why these studies are important. First, as a pediatric bone marrow transplant physician, I am keenly aware that profound clinical problems remain for my patients. Matched stem cells from healthy donors are often unavailable and poor recovery of the immune system after transplantation results in an unacceptably high incidence of death and illness from infection. Second, as a stem cell biologist I recognize that the well established tools that can be applied specifically to hematopoietic development from hESC are uniquely able to answer some of the most fundamental questions about how hESC generate tissues and how we can best control the process. With these answers we will be able to tailor our approaches for differentiation to all tissue types and move the intriguing biology of hESC more rapidly and safely to the clinic.

Constructing a fate map of the human embryo
University of California, San Francisco
Principal Investigator: Susan J. Fisher, Ph.D. Comprehensive Research Grant Program

The United States government does not fund research involving human embryos or cells that were grown from them after August 9, 2001. In addition, other restrictions have been imposed that make these types of experiments extremely difficult to do. For example, work cannot be conducted alongside research that is funded by government agencies, the typical mode in which academic research laboratories operate. In practical terms, this means that duplicate facilities must be created to do the large amount of research that is needed to turn human embryonic stem cells (hESCs) into robust experimental tools that will enable us to understand disease processes, the first step in curing them. These onerous regulations, unprecedented in our country, have stifled progress in this exciting new area of medical research. Thus, there is a great deal of basic work that remains to be accomplished. Our group is focusing on one particular area--the enigmatic process that occurs when an embryo--which would otherwise be discarded at the conclusion of an in vitro fertilization (IVF) treatment--is donated for research and grown in a laboratory. In certain cases, the cells that would have gone on to form specialized tissues such as blood cells, and major organs such as the heart and pancreas, continue to make copies of themselves. As first shown in 1998, the copies, termed hESC lines, may remember how to do their original job, i.e., differentiate into every type of cell in the human body. Scientists think that this is possible, because in many laboratory animals the equivalent populations retain this ability. Our group wants to optimize the methods that are used to make new hESC lines, because the techniques that are currently used are essentially random. Embryos are maintained in the laboratory until outgrowths--collections of cells that look very different from one another--appear. During this 2- to 3-week process, many of these cells die, but a subset start to make copies of themselves. Thus, much remains to be learned about the derivation process. For example, we do not know when, during this extended time period, the actual progenitor cell(s) arises, and it is unclear whether all the cells of the embryo are equally able to give rise to hESC lines. Thus, we propose to test the theory that there are better, more controlled ways to produce hESCs. Recently, our collaborators showed that it is possible to make lines from single cells that are removed from human embryos at a specific time. We want to use their method to determine if hESCs made from individual cells that are removed at different times from specific regions of the embryo are better equipped to generate all the cell types found in the body. Essentially, we want to harness and standardize the process of developing new lines. This work, which cannot be supported by the federal government, has important implications for devising hESC-based patient therapies.

Understanding hESC-based Hematopoiesis for Therapeutic Benefit
University of California, San Francisco
Principal Investigator: Andrew D. Leavitt, M.D. Comprehensive Research Grant Program

Hematopoietic stem cell transplantation is the treatment of choice for many hematologic malignancies, and it is used to treat an expanding number of congenital blood disorders. However, only ~30% of patients who can benefit from this treatment have a matched sibling that can serve as the ideal donor. While the national marrow donor program and umbilical cord blood programs provide unrelated donor cells to many patients lacking a sibling donor, a large percentage of patients remain without a suitable donor, leaving them with suboptimal therapeutic options. This problem is more severe in certain ethnic populations, including people of Latino and Asian descent, groups that constitute a large part of California's population. New sources of therapeutic hematopoietic stem cells are therefore needed.

Human embryonic stem cells, with their unlimited self-renewal capacity and their ability to generate all human cell types, provide a novel and exciting opportunity to obtain hematopoietic stem cells, thereby filling a critical therapeutic void. However, many hurdles remain before this vision can be realized,

including the identification of more optimal human embryonic stem cell lines and better methods to direct the development of specific cell types from embryonic stem cells.

This proposal seeks to shed new insight into how we might better control and direct the development of human embryonic stem cells into therapeutically useful hematopoietic stem cells that can be used for transplantation. Our effort focuses on understanding how a specific class of small RNAs, called microRNAs, regulates the differentiation of human embryonic stem cells into specific cell types. We aim to uncover the identity of microRNAs that are important for this process, which will serve as useful biomarkers, or guides, for evaluating the therapeutic suitability of existing and newly derived human embryonic stem cell lines. In addition, we will develop techniques and reagents to modulate the expression of these critical small molecules to help direct human embryonic stem cell development for clinical therapeutic utility.

Guiding the developmental program of human embryonic stem cells by isolated Wnt factors

Stanford University
Principal Investigator: Roel Nusse, Ph.D. Comprehensive Research Grant Program

Just like cells in a human embryo, embryonic stem cells have the potential to give rise to all cell types and tissues in a human body. That is why it is an exciting prospect to use these cells in tissue repair. But in order to do so, we have to understand how we can guide the differentiation of stem cells. For example, if one wants to use stem cells for replacing defective insulin-producing cells in the pancreas, we have to learn how we can convert stem cells into pancreas cells, or at least precursors to pancreas cells.

So the question is then, how do cells in an embryo become different from each other? Research done in animals has shown that there are signaling proteins that instruct cells to change from one type into another. One important group of these signaling proteins are the Wnts. Studied in our lab for along time, Wnts are powerful differentiation factors. To use Wnt proteins as factors under controlled conditions, one has to be able to isolate them. This has been a major problem in the past, but we have solved this recently. We are therefore now in a position to test how Wnt proteins, when added to stem cells, change the state of differentiation of the cells and our preliminary results indicate that there are indeed significant consequences.

A second question we want to address is how we can recognize intermediate stages in stem cell differentiation. Going from a stem cell to a pancreatic insulin-producing cell is a step-wise process, following a road map where we know the beginning and the end but not the steps in between. We intend to perform gene-chip experiments to chart those steps and to map the pathways that stem cells follow to differentiated progeny cells.

Finally, we will focus our research on promoting the differentiation of stem cells into endoderm, a tissue that is the precursor to pancreas development. We expect this research to contribute significantly to our insights into stem cell behavior, but also to generate new tools to improve the use of stem cells for regenerative medicine.

microRNA Regulation of Cardiomyocyte Differentiation from Human Embryonic Stem Cells

The J. David Gladstone Institutes
Principal Investigator: Deepak Srivastava, M.D. Comprehensive Research Grant Program

Regenerative therapies could be particularly beneficial for heart disease, which is the leading killer of adults in the U.S, and is responsible for the 5 million Americans with insufficient cardiac function. At the other end of the age spectrum, malformations of the heart involving abnormal cell lineage or morphogenetic decisions are the leading noninfectious cause of death in children. Unfortunately, since adult heart cells cannot multiply after birth, the heart has almost no regenerative capacity after injury or in response to malformations. Deciphering the secrets of heart formation might lead to novel approaches to repair or regenerate damaged heart muscle using embryonic stem cells (ESCs) and progenitor cells. Our research is focused on determining what causes ESCs to specialize into cells that belong to the mesodermal, or middle, layer of an embryo, which develops into blood, muscle, and bone, among other cells, with a specific focus on cues that stimulate cardiac and skeletal muscle formation. Small RNA molecules called microRNAs have emerged as an elegant and novel mechanism nature uses to titrate dosage of critical proteins by regulating the flow of genetic information as it is translated into proteins. microRNAs are active dynamically and specifically in developing cardiac and skeletal muscle during muscle formation. In mice and flies, microRNAs regulate the balance of muscle formation vs. expansion of progenitor cells. We have evidence that microRNAs can control mouse embryonic stem cells (mESCs) and can promote formation of mesoderm and inhibit formation of other cell types such as brain or gut cells. This may be true in human ESCs also. However, NIH-approved human ESC (hESC) lines are contaminated with mouse feeder cells, are difficult to disperse into single cells and do not grow robustly enough to generate homogeneous pools of genetically altered cells. This has made it difficult to generate homogenous population of cells that could be used for discovery and future potential therapeutic applications. The aims of this grant will use non-NIH approved lines to meet these objectives and are not fundable by the NIH. We hypothesize that specific microRNAs influence early mesoderm commitment and later steps of myogenic expansion or formation from hESCs by controlling other key regulatory events. To test this hypothesis, we propose three specific aims: 1) Determine if microRNAs can promote mesoderm formation and subsequent decisions of cardiac muscle proliferation or differentiation in hESCs; 2) Determine if specific microRNAs repress other lineages in hESCs; 3) Determine the mechanisms by which microRNAs regulate mesoderm commitment, muscle differentiation and proliferation. The tools and understanding developed here will ultimately be used to generate myocytes either directly or through subsequent screens for drugs targeted at the pathways discovered by the proposed work.

GENOMIC INSTABILITY AND CANCER

The utility of embryonic stem cells as a therapy depends on the ability of these remarkable cells to divide, self-renew and still mature into many different cell types. Continuous culture outside the body, normal aging, and disease all can result in the accumulation of damage to the DNA of stem cells. The resulting genetic instability can impair stem cell growth and differentiation, and render the cells useless. Even worse, genomic instability can transform the stem cells into cancerous cells capable of forming tumors. Little is known about the biology of human embryonic stem cells and precisely which mechanisms are important for protecting the stability of their genome, although clearly this information is needed to develop stem cells as a safe therapy. In 2007, eight awards are approved for funding that focus on unraveling the molecular mechanisms that protect and repair the DNA of human embryonic stem cells.

LEON J. THAL SEED GRANT PROGRAM

The APOBEC3 Gene Family as Guardians of Genome Stability in Human Embryonic Stem Cells
The J. David Gladstone Institutes
P.I.: Warner C. Greene, M.D., Ph.D.
$780,000 for 2 years

Screening for Oncogenic Epigenetic Alterations in Human ES Cells
University of Southern California
P.I.: Peter William Laird, Ph.D., Sc.D.
$685,000 for 2 years

Sources of Genetic Instability in Human Embryonic Stem Cells.
City of Hope National Medical Center
P.I.: Timothy R. O'Connor, Ph.D.
$360,000 for 2 years

Functions of RB family proteins in human embryonic stem cells
Stanford University
P.I.: Julien Sage, Ph.D.
$525,000 for 2 years

Role of the tumor suppressor gene, p16INK4a, in regulating stem cell phenotypes in embryonic stem cells and human epithelial cells
University of California, San Francisco
P.I.: Thea D. Tlsty, Ph.D.
$640,000 for 2 years
Also referenced in: Self-renewal

In Vivo Imaging of Human Embryonic Stem Cell Derivatives and Tumorigenicity
Stanford University
P.I.: Joseph C. Wu, M.D., Ph.D.
$660,000 for 2 years
Also referenced in: New Stem Cell Lines and Technologies

Dollar amount shown is the amount requested rounded to the nearest $5,000, not the amount awarded.

COMPREHENSIVE RESEARCH GRANT PROGRAM

The Dangers of Mitochondrial DNA Heteroplasmy in Stem Cells Created by Therapeutic Cloning
University of California, Irvine
P.I.: Douglas C. Wallace, Ph.D.
$2,530,000 for 4 years
Also referenced in: New Stem Cell Lines and Technologies

Mechanisms to maintain the self-renewal and genetic stability of human embryonic stem cells
University of California, San Diego
P.I.: Yang Xu, Ph.D.
$2,570,000 for 4 years
Also referenced in: Self-renewal

Dollar amount shown is the amount requested rounded to the nearest $5,000, not the amount awarded.

ABSTRACTS

[Provided by applicant]

The APOBEC3 Gene Family as Guardians of Genome Stability in Human Embryonic Stem Cells

The J. David Gladstone Institutes
Principal Investigator: Warner C. Greene, M.D., Ph.D. Leon J. Thal SEED Grant Program

The successful use of human embryonic stem cells (hESCs) as novel regenerative therapies for a spectrum of currently incurable diseases critically depends upon the safety of such cell transfers. hESCs contain roughly 3 million "jumping genes" or mobile genetic retroelements that comprise up to 45% of their genetic material. While many of these retroelements have been permanently silenced during evolution by crippling mutations, many remain active and capable of moving to new chromosomal locations potentially producing disease-causing mutations or cancer. More mature differentiated cells control retroelement movement (retrotransposition) by methylating the DNA comprising these elements. Strikingly, such DNA methylation is largely absent in hESCs because these cells must be able to develop into a wide spectrum of different tissues and organs. Thus, in order to protect the integrity of their genomes, hESCs must deploy an additional defense to limit retroelement retrotransposition. Recent studies of HIV and other exogenous retroviruses have identified the APOBEC3 family of genes (A3A-A3H) as powerful anti-retroviral factors. These APOBEC3s interrupt the conversion of viral RNA into DNA (reverse transcription), a key step also used by retroelements for their successful retrotransposition. We hypothesize that one or more of the APOBECs function as guardians of genome integrity in hESCs. We propose to compare and contrast which APOBEC3s are expressed in one federally approved and nine nonapproved hESC lines and to assess the natural level of retroelement RNA expression occurring in each of these lines. Next we will test whether the knockdown of expression of these APOBEC3s in the hESCS lines by RNA interference leads to a higher frequency of retrolement retrotransposition. Finally, if higher levels of retrotransposition are detected, we will examine whether these cells display an impaired ability to differentiate into specific tissue types corresponding to the three germ cell layers (ectoderm, mesoderm, and endoderm) and whether increased retrotransposition is associated with a higher frequency of malignant transformation within the hESC cultures. These studies promise to provide important new insights into how genomic stability in is maintained in hESCs and could lead to the identification of specific GMP culture conditions that minimize the chances of such unwanted retrotransposition events in cells destined for infusion into patients. These studies are directly responsive to the CIRM request for application. If funded, these studies would allow the entry of my laboratory with extensive APOBEC experience, into the exciting field of stem cell biology.

Screening for Oncogenic Epigenetic Alterations in Human ES Cells

University of Southern California
Principal Investigator: Peter William Laird, Ph.D., Sc.D. Leon J. Thal SEED Grant Program

Embryonic stem cell-based therapies hold great promise for the treatment of many human diseases. These therapeutic strategies involve the culture and manipulation of embryonic stem cells grown outside the human body. Culture conditions outside the human body can encourage the development of changes to the cells that facilitate rapid and sustained cell growth. Some of these changes can resemble abnormal changes that occur in cancer cells. These include "epigenetic" changes, which are changes in the structure of the packaging of the DNA, as opposed to "genetic" changes, which are changes in the DNA sequence.

Cancer cells frequently have abnormalities in one type of epigenetic change, called "DNA methylation". We have found that cultured embryonic stem cells may be particularly prone to develop the type of DNA methylation abnormalities seen in cancer cells. A single rogue cell with DNA methylation abnormalities

predisposing the cell to malignancy can jeopardize the life of the recipient of stem cell therapy. We have developed highly sensitive and accurate technology to detect DNA methylation abnormalities in a single cell hidden among 10,000 normal cells.

In this seed grant, we propose to screen DNA methylation abnormalities at a large number of genes in different embryonic stem cells and compare their DNA methylation profiles to normal and cancer cells. This will allows us to identify the dangerous DNA methylation abnormalities most likely to occur in cultured embryonic stem cells. We will then develop highly sensitive assays to detect these DNA methylation abnormalities, using our technology. We will then use these assays to determine ES cell culture conditions and differentiation protocols most likely to cause these DNA methylation abnormalities to arise in cultured ES cells.

The long-term benefits of this project include 1) an increased understanding of the epigenetics of human embryonic stem cells, 2) insight into culture conditions to avoid the occurrence of epigenetic abnormalities, and 3) a technology to monitor for epigenetic abnormalities in ES cells intended for introduction into stem cell therapy patients.

Sources of Genetic Instability in Human Embryonic Stem Cells.

City of Hope National Medical Center
Principal Investigator: Timothy R. O'Connor, Ph.D. Leon J. Thal SEED Grant Program

The constant exposure of cells to endogenous and exogenous agents that inflict DNA damage requires active repair processes to eliminate potentially mutagenic events in stem cells leading to cancer. The same agents menace early human embryos with DNA damage that can ultimately lead to mutations, cancer, and birth defects. In vitro, human embryonic stem cells (HESCs) spontaneously undergo events leading to genetic instability and mutations. All these three types of genetic problems can have similar links to malfunctions in DNA repair systems, but little information now exists for HESCs. Therefore, the first step in understanding the causes of HESC genetic instability is to understand which DNA repair systems are defective. We will investigate the basis for this phenomenon in HESCs by evaluating their capacity to either repair DNA or form mutations. First, we will culture two HESC lines and compare HESC repair and mutation formation to that of control cells. We will use a new technique which simplifies the production and use of the feeder cells that support the growth of the HESCs. We will also test the genetic stability of HESCs grown on conventional feeder cells, as well as those grown in feeder free culture. We will use three types of DNA repair assays to monitor the genetic stability of the two HESC lines grown in these different ways. In the first of these assays, DNA molecules with different randomly-induced damage are transferred into HESCs, and DNA repair is followed by the re-establishment of the activity of a reporter protein that is coded for in the damaged DNA. A second assay will introduce specific DNA damage at a unique site in DNA that is transferred to HESCs and repair is determined using a polymerase chain reaction-based technique. Since aneuploidy is also known to be caused by double-strand DNA breaks, we will use two other assays to evaluate capacity of HESCs to repair that type of damage. These experiments will indicate if DNA repair pathways that eliminate DNA damage are dysfunctional and cause genetic instability. The final endpoint for these preliminary experiments is the formation of mutations. To study this, we have modified an assay system so that it will function in normal human cells to monitor mutations which arise spontaneously or those which are induced by various agents. In summary, these investigations will provide the basis for understanding genetic instability in HESCs that can direct cells to tumorgenic outcomes. The employment of HESCs clinically will require such knowledge. Moreover, these results will also yield information on susceptibility to mutations of cells early in development. The practical and basic science aspects of this seed grant proposal should lead to a complete proposal in the near future.

Functions of RB family proteins in human embryonic stem cells
Stanford University
Principal Investigator: Julien Sage, Ph.D. Leon J. Thal SEED Grant Program

Nearly one out of every two Californians born today will develop cancer at some point in their lives, and it is likely that one in five persons will die of the disease. We propose to study the mechanisms of action of the RB gene, which is mutated in a broad range of human cancers, including pediatric cancers of the eye and the bone, and adult tumors such as lung, breast, prostate and liver cancers. RB normally acts as a tumor suppressor. When RB is mutated, cells lose the ability to sense when to cycle or not and they divide too much, thereby initiating cancer. Because RB is mutated in so many human cancers, therapies that could re-introduce RB function in cancer cells would benefit a great number of cancer patients.

A key question is to determine in which cell type loss of RB function is most detrimental. Knowing the answer to this question would help to diagnose cancer early and target specific cells within tumors, making treatment more effective. Recent evidence suggests that loss of RB may initiate cancer in stem cells . Because human embryonic stem cells (hESCs) give rise to any other stem cells, we will study the role of RB in hESCs. The results of these experiments will thus be applicable to a broad range of human cancers.

Specifically, we will use novel tools that will allow us to precisely alter RB levels in hESCs. We will then study the consequences of these manipulations for the proliferation of these cells; lower levels of RB may promote proliferation, while higher levels of RB may slow proliferation and push these embryonic stem cells to become more mature. We will then investigate the molecular mechanisms underlying these observations, beginning with the cellular pathway leading to retinal development because of RB's involvement in retinal cancer.

Because RB is usually deleted in cancer cells, there is no simple way to re-express RB function in these cells. However, two genes related to RB, p107 and p130, are rarely deleted in cancers and can compensate for loss of RB in mouse cells. Therefore, we will also study the role of p107 and p130 in hESCs, to determine if the functions of these two genes also overlap with RB function in these human cells and their progeny. If this is the case, knowing how to control the expression of p107 and p130 in hESCs may result in the development of a novel therapeutic strategy against human cancers associated with loss of RB.

A better knowledge of the cells from which cancer arises and of the molecular mechanisms by which cancer initiates will lead in the future to the development of novel means to diagnose cancer earlier, thereby increasing the chances of a successful therapy.

In addition, because of the central role of RB family members in multiple cellular functions, these experiments in hESCs may provide novel insight into the basic biology of these stem cells, which will eventually allow us to manipulate these cells more efficiently to treat a broad range of human diseases.

Role of the tumor suppressor gene, p16INK4a, in regulating stem cell phenotypes in embryonic stem cells and human epithelial cells.
University of California, San Francisco
Principal Investigator: Thea D. Tlsty, Ph.D. Leon J. Thal SEED Grant Program
Also referenced in: Self-renewal

The roles of stem cells are to generate the organs of the body during development and to stand ready to repair those organs through repopulation after injury. In some cases these properties are not correctly regulated and cells with stem cell properties expand in number. Recent work is demonstrating that the

genes that control stem cell properties are sometimes the same genes that are mutated in cancer. This means that a cell can simultaneously acquire stem cell properties and cancer properties. In order to effectively use stem cells for therapeutic purposes we need to understand the link between these two programs and devise ways to access one program without turning on the other. In other words, we would like to expand stem cell populations without them turning into cancer.

Recent work in our laboratory has found that the reduction of a specific tumor suppressor gene, p16, not only removes an important barrier to cancer but also confers stem cell properties within the cell. Cells that have reduced p16 activity can turn on a program that increases and reduces expression of specific genes that control differentiation. In this proposal we will test whether the continued reduction of this tumor suppressor gene creates human embryonic stem cells (hESC) that are unable to differentiate. We hypothesize that the lack of p16 represses multi-lineage potential by activating an epigenetic program and silencing genes that drive differentiation. To test this hypothesis we will first determine if lack of p16 activity is necessary for hESCs to develop into different cell types. Second, we will determine if continued lack of p16 activity is sufficient to inhibit differentiation of hESCs. Finally, we will determine if transient lack of p16 activity is sufficient for a non-stem cell to exhibit properties of a stem cell after propagation in a stem cell niche.

Since these types of events are potentially reversible, targeting such events may become clinically useful. These new observations identify novel opportunities. They provide potential markers for determining if someone is susceptible to cancer, as well as, providing potential targets for prevention and therapy. We hypothesize that these properties are critically relevant to the formation of cancer and will provide insights into the role of epigenetic modifications in disease processes and stem cell characteristics.

In Vivo Imaging of Human Embryonic Stem Cell Derivatives and Tumorigenicity
Stanford University
Principal Investigator: Joseph C. Wu, M.D., Ph.D. Leon J. Thal SEED Grant Program
Also referenced in: New Stem Cell Lines and Technologies

Human embryonic stem cells (hESCs) are one of the most fascinating subjects of interest in all of biology and medicine these days. Under certain physiologic conditions, they can be induced to become specialized cells such as brain, cardiac, liver, pancreatic, and bone marrow cells. This opens up the exciting possibility that hESCs may one day be used to treat patients with Parkinson's disease, heart conditions, hepatitis, diabetes, and leukemia, just to name a few currently intractable diseases that affect millions of Americans alone. This field of cell-based therapies to treat human diseases is generally referred to as "regenerative medicine."

Scientists who want to study hESCs or their specialized cell derivatives typically inject them into small animal models such as mice and rodents. However, researchers currently are unable to monitor noninvasively these cells after transplant. Instead, these animals are typically sacrificed for postmortem biopsy, which precludes long-term follow-up of transplanted cells. Without the ability to follow the progress of transplanted cells over a longer period of time, important insights into hESC fates in vivo have not been forthcoming. Thus, developing a novel technology to track transplanted hESCs and their specialized cell derivatives would represent a major advancement in this field that will produce wide-ranging theoretical and practical implications.

Another problem with transplantation of hESCs is the potential to cause teratomas. Teratomas are disorganized arrays of cell differentiation that appear to recapitulate many of the events involved in early embryonic development. Clearly, the teratoma formation risk is a major obstacle to future clinical application of hESCs. In this proposal, we will evaluate how teratoma forms in living subjects over time using the imaging techniques that we have developed as well as how best to prevent them in the first place.

Due to the serious risks posed by teratoma formation, it is necessary to induce hESCs to become specialized cell derivatives first before transplantation for therapeutic purposes. However, this process is not efficient at present despite intense efforts searching for methods to expedite it. Our team plans to tackle this problem using the latest genomics and proteomics technology.

In summary, our proposal is a targeted response to the CIRM SEED grant. It seeks to develop a novel technology (molecular imaging) that will address a critical barrier in clinical translation of hESC therapy (teratoma formation) and provide a better understanding of cardiac cell differentiation process (genomics/proteomics). Our well-established multidisciplinary team has the required training, experience, and innovation to complete the project. Overall, we are confident that our proposed studies will generate significant progress in this field, in both scientific knowledge and useful therapies.

The Dangers of Mitochondrial DNA Heteroplasmy in Stem Cells Created by Therapeutic Cloning

University of California, Irvine

Principal Investigator: Douglas C. Wallace, Ph.D. Comprehensive Research Grant Program

Also referenced in: New Stem Cell Lines and Technologies

In therapeutic cloning, a patient's cell is combined (fused) to an enucleated donated egg (oocyte) from an unrelated woman or from another animal. It is hoped that cellular factors in the egg cytoplasm will reprogram the patient's cell nucleus making it capable of generating replacement cells for the patient's body. Thus, if a patient is suffering from Parkinson Disease due to loss of brain cells, these cells could be replaced with differentiated, individualized, nuclear-transplantation, embryonic stem (ntES) cells. While this strategy should generate ntES cells with the patient's nuclear DNA (nDNA), it overlooks the fact that another part of the cell, the mitochondrion, also has DNA, the mitochondrial DNA (mtDNA). While the nDNA contains the blueprints for assembling the structure of the cell and body, analogous to carpenter's plans for a house; the mtDNA contains the blueprints for the cellular electrical system, the wiring diagram of the house. It is universally agreed that mixing the nDNAs from two different cells would be destructive, yet the potentially disastrous effects of mixing different mtDNAs has been overlooked. In electricity, randomly mixing the components of two different integrated electrical circuits will result in short circuits. The same appears to be true for the cell. In mice in which we artificially mixed two mtDNAs, the resulting mice aged and died prematurely, had a striking increased frequency of cancer, and an increased mtDNA mutation rate. Moreover, in human studies, the accumulation of mtDNA mutations has been associated with aging and the development of cancer. Therefore, to document what happens to the mtDNAs during the creation and growth of htES and hES cells, we propose to create ntES cells by fusion of human cells to enucleated rabbit eggs and then to monitor the fate of the human and rabbit mtDNAs. We will also determine if the mitochondria and mtDNAs of hES cells can influence the differentiated state of tissue cells, investigate the nature and extent of mtDNA mutations that accumulate in ES cells, and determine if mixing different mtDNAs in cells is deleterious. Then we will determine the effects of these various mtDNA genetic factors on the power output of the mitochondria and its effects on the ES cell's capacity to differentiate and potentially to form tumors. Finally, we propose to develop a series of procedures to control the origin and nature of the mtDNAs in ntES and hES cells and to use changes in mitochondrial function to regulate ntES and hES cell growth and regulation. It is our hope that by utilizing mitochondrial biology and genetics it may be possible to develop strategies for creating individualized stem cells without using donated oocytes.

Mechanisms to maintain the self-renewal and genetic stability of human embryonic stem cells

University of California, San Diego
Principal Investigator: Yang Xu, Ph.D. Comprehensive Research Grant Program
Also referenced in: Self-renewal

Human embryonic stem cells (hESCs) are capable of unlimited self-renewal, a process to reproduce self, and retain the ability to differentiate into all cell types in the body. Therefore, hESCs hold great promise for human cell and tissue replacement therapy. Because DNA damage occurs during normal cellular proliferation and can cause DNA mutations leading to genetic instability, it is critical to elucidate the mechanisms that maintain genetic stability during self-renewal. This is the overall goal of this proposal. Based on our recent findings, I propose to investigate two major mechanisms that might be important to maintain genetic stability in hESCs. First, I propose to elucidate pathways that promote efficient DNA repair in hESCs. Second, based on our recent findings, I hypothesize that another primary mechanism to maintain genetic stability in self-renewing hESCs is to eliminate DNA-damaged hESCs by inducing their differentiation. Therefore, I propose to identify the pathways that regulate the self-renewing capability of hESCs in the presence and absence of DNA damage. In summary, the proposed research will contribute significantly to our understanding of the pathways important to maintain self-renewal and genetic stability in hESCs. This information will provide the foundation to improve the culturing condition of hESCs to promote efficient self-renewal with minimum genetic instability, a prerequisite for the development of hESCs into human therapeutics.

One major objective of the proposed research is to improve the genetic manipulation technologies in hESCs, including transgenic and gene targeting technologies. While mouse models are valuable tools to study the mechanisms of the pathogenesis in human diseases, many differences between mouse and human cells can lead to distinct phenotypes as well as the common phenomenon that certain therapeutic interventions work well in mouse models but poorly in humans. Therefore, it is of high priority to create disease-specific hESCs as powerful genetic tools to study the mechanism of the pathogenesis in human diseases. In addition, the unlimited supply of primary cells derived from the disease-specific hESCs will become valuable reagents for drug discovery. There are two ways to generate the disease-specific hESCs. One approach is through nuclear transfer that has been proven extremely difficult in human context and so far unsuccessful. The other is to employ the transgenic and gene targeting techniques to create disease-specific hESCs. Therefore, the proposed research will significantly improve our capability to generate disease-specific hESCs. After experimenting with various existing hESC lines, we found that only the non-federally-approved hESC lines developed recently at Harvard University is most suitable for genetic manipulation technologies. Since the research involving the HUES lines can not be supported by federal government, CIRM is in a unique position to support this proposed research.

NEW STEM CELL LINES AND TECHNOLOGIES

The successful development of novel stem cell-based therapies requires new technologies and cell lines. Challenges to therapy development include finding effective ways to turn on desired genes in stem cells, avoiding immune rejection of grafted cells and tissues that are derived from stem cells, and tracking the fate and function of these cells after transplantation. One way to avoid immune rejection of transplanted cells is to use embryonic stem cells that have been 'reprogrammed' to match the patient's own cells. Reprogramming techniques also can be used to generate disease-specific embryonic stem cell lines by using nuclei from patients with the genetic background(s) at risk for disease or that would lead to disease. These new lines could be used to study the mechanism of the disease, and as a screening tool to hunt for new drugs for treatment. After stem cells are introduced into a living organism, tracking their fate and function in the body is critical to determine if the cells integrated successfully where needed, and if they behaved in an unexpected or adverse manner. In 2007, nine awards are approved for funding that focus on generating new and improved human embryonic stem cell lines, on pioneering new methods to monitor stem cell fate following transplant, or on developing methods to manipulate gene expression in human embryonic stem cells.

LEON J. THAL SEED GRANT PROGRAM

Labeling of human embryonic stem cells with iron oxide nanoparticles and fluorescent dyes for a non-invasive cell depiction with MR imaging and optical imaging
University of California, San Francisco
P.I.: Heike E. Daldrup-Link, M.D., Ph.D.
$255,000 for 1 year

Novel vectors for gene transfer into human ES cells
Stanford University
P.I.: Mark A. Kay, M.D., Ph.D.
$645,000 for 2 years

Patient-specific cells with nuclear transfer
Stanford University
P.I.: Seung K. Kim, M.D., Ph.D.
$660,000 for 2 years

Production of Oocytes from Human ES Cells
University of California, Irvine
P.I.: Grant R. MacGregor, Ph.D.
$625,000 for 2 years
Also referenced in: Pluripotency and Differentiation

Induction of pluripotency in fibroblasts by fusion with enucleated human embryonic stem cell syncytia
University of California, San Francisco
P.I.: Holger Willenbring, M.D.
$345,000 for 2 years

In Vivo Imaging of Human Embryonic Stem Cell Derivatives and Tumorigenicity
Stanford University
P.I.: Joseph C. Wu, M.D., Ph.D.
$660,000 for 2 years
Also referenced in: Genomic Instability and Cancer

Dollar amount shown is the amount requested rounded to the nearest $5,000, not the amount awarded.

COMPREHENSIVE RESEARCH GRANT PROGRAM

Functional Genomic Analysis of Chemically Defined Human Embryonic Stem Cells
Stanford University
P.I.: Julie C. Baker, Ph.D.
$2,630,000 for 4 years

Human oocyte development for genetic, pharmacological and reprogramming applications
Stanford University
P.I.: Renee A. Reijo Pera, Ph.D.
$2,470,000 for 4 years

The Dangers of Mitochondrial DNA Heteroplasmy in Stem Cells Created by Therapeutic Cloning
University of California, Irvine
P.I.: Douglas C. Wallace, Ph.D.
$2,530,000 for 4 years
Also referenced in: Genomic Instability and Cancer

Dollar amount shown is the amount requested rounded to the nearest $5,000, not the amount awarded.

ABSTRACTS

[Provided by applicant]

Labeling of human embryonic stem cells with iron oxide nanoparticles and fluorescent dyes for a non-invasive cell depiction with MR imaging and optical imaging

University of California, San Francisco
Principal Investigator: Heike E. Daldrup-Link, M.D., Ph.D. Leon J. Thal SEED Grant Program

Non-invasive imaging techniques for an in vivo tracking of transplanted stem cells offer real-time insight into the underlying biological processes of new stem cell based therapies, with the aim to depict stem cell migration, homing and engraftment at organ, tissue and cellular levels. We showed in previous experiments, that stem cells can be labeled effectively with contrast agents and that the labeled cells can be tracked non-invasively and repetitively with magnetic resonance imaging (MRI) and Optical imaging (OI). The purpose of this study is to apply and optimize these labeling techniques for a sensitive depiction of human embryonic stem cells (hESC) with OI and MRI.

Experimental Design: hESC will be labeled with various contrast agents for MRI and OI, using a variety of labeling techniques, different contrast agent concentrations and different labeling intervals (1h - 24h). The cellular contrast agent uptake will be proven by mass spectrometry (quantifies the iron oxides) and fluorescence microscopy (detects fluorescent dyes). The labeled hESC will undergo imaging studies and extensive studies of their viability and ability to differentiate into specialized cell types.

Imaging studies: Decreasing numbers of 1 x 10^5 - 1 x 10^2 contrast agent-labeled hESC and non-labeled controls will be evaluated with OI and MRI in order to determine the best contrast agent and labeling technique as well as the minimal detectable cell number with either imaging technique. In addition, samples of hESC will be investigated with OI and MRI at 1 min, 2 min, 5 min, 1h, 2h, 6h, 12h, 24h and 48 h in order to investigate the stability of the label over time.
Viability and differentiation assays of the hESC will be performed before and after the labeling procedure in order to prove an unimpaired viability and function of the labeled cells.

Image analysis and histopathology: For quantitative analyses, MR signal intensities and mean fluorescence signal intensities of the cell samples and the image background will be measured and compared for significant differences between different groups (labeled cells and non-labeled controls, different contrast agents, labeling techniques, different cell numbers, different time points after labeling) using dedicated statistical tests. These quantitative data will be compared with results from mass spectrometry and histopathology.

Significance: The derived data should establish and optimize hESC labeling with contrast agents for a non-invasive depiction of the labeled cells with MR and OI imaging techniques. Our method would be in principle readily applicable for monitoring of hESC -based therapies and direct correlations between the presence and distribution of hESC in the target organ and functional improvements. The results of this study will be the basis for subsequent NIH grant applications.

Novel vectors for gene transfer into human ES cells
Stanford University
Principal Investigator: Mark A. Kay, M.D., Ph.D. Leon J. Thal SEED Grant Program

Human embryonic stem cells have a great potential for medical therapeutics. However, the genes required for altering the fate of these cells to differentiate into a particular tissue or cell type is not well understood. The ability to efficiently transfer genes or silence genes in ES cells would be of great benefit for two reasons: 1) A combination of gene therapy and ES stem cells will likely broaden the therapeutic potential of these cells, and 2) the ability to alter gene expression will provide important tools for unraveling the genetic programs required for targeted differentiation into a specific tissue or cell-type. Viral gene transfer vectors are derived from viruses. The viral genes are removed and replaced with a therapeutic gene or a gene under biological study. Thus, the viral shell is used to transfer the desired gene into cells. Although there has been some success, current gene transfer vectors do not work efficiently in ES cells. To make this new gene transfer vector, we will use the adenoassociated virus (AAV). This virus is not associated with any known disease and has been used in a number of human clinical trials. Another advantage of vectors based on these viruses is that they can be used to overproduce a gene product, turn off a gene product, and even target a mutation in a specified gene. We plan to use a recent strategy developed in our laboratory to select for AAV viruses that are very efficient at infecting human ES cells. Once these viruses are identified, as a proof-of-concept, we will make gene transfer vectors and test them in new human ES cells for their ability to over-express and turn off genes that we believe are important in early human ES cell differentiation.

Patient-specific cells with nuclear transfer
Stanford University
Principal Investigator: Seung K. Kim, M.D., Ph.D. Leon J. Thal SEED Grant Program

Somatic cell nuclear transfer (NT) is a powerful research tool with the potential for creating unique cell and tissue sources for studies of disease pathogenesis and regenerative medicine. Creation of pluripotent mouse embryonic stem (ES) cells using NT has been achieved and the prospects for generating human ES cells by NT are promising. However, there are only a handful of researchers who have reported their experience with NT and development of this approach in California would benefit from increasing dedicated efforts toward this goal. We have assembled a team focused on NT that has achieved several experimental milestones that motivate these proposed studies of NT in human oocytes. These prior achievements include NT in mouse oocytes, efficient production of novel ES cells from mouse embryos, and controlled enucleation of recipient human oocytes. With CIRM SEED funds, we will use systematic approaches to identify conditions that generate multipotent human cells from NT into human oocytes, with the goal of eventually producing new patient-specific human ES cell (hESC) lines for studies of disease pathogenesis, transplantation and tissue regeneration.

Successful outcomes from this proposal would enable us and others to generate new ES cell lines to study the pathogenesis of human diseases. Discovery of molecular mechanisms underlying diseases inevitably will produce novel strategies for diagnosis, prognosis or therapeutics. For instance investigators here have provided evidence for dysregulated signaling through the NFAT/calcineurin pathway in Down syndrome (DS), which arises in patients with trisomy for chromosome 21. Development of diagnostics or therapies that exploit the tenets of this model would surely be accelerated by tests of the model with embryonic human tissue harboring the classic trisomy 21 karyotype. Currently, such embryonic tissues for experimental studies are not available; we postulate that a human DS ES cell line generated by NT could be used to develop differentiating embryonic human tissues for study of dysregulated signaling in vitro. Similar logic would justify generating patient-derived ES cell lines to produce experimental systems for studies of other diseases, including childhood congenital malformations, sickle cell disease, or neurological disorders lacking models or a defined pathophysiologic basis, like amyotropic lateral sclerosis (ALS).

Production of Oocytes from Human ES Cells
University of California, Irvine
Principal Investigator: Grant R. MacGregor, Ph.D. Leon J. Thal SEED Grant Program
Also referenced in: Pluripotency and Differentiation

The ability of human embryonic stem (hES) cells to form a wide variety of adult human cell types offers hope for development of novel therapies to treat human degenerative diseases such as Alzheimer's, diabetes, and muscular dystrophy. However, to prevent rejection of the transplanted cells by a patient's immune system it will be important to use hES cell derived tissues that are immunologically matched to the patient. One way to do this involves somatic cell nuclear transplantation (SCNT) where the nucleus containing the genetic information is transferred from a patient's cell into a human oocyte (egg) from which the nucleus has been removed. The oocyte is then stimulated to divide into a small group of cells from which new hES cells are derived. As cells derived from these hES cells contain the patient's DNA they will be immune-matched to the patient, thereby preventing tissue-rejection.

While SCNT has been performed using cells and eggs from mice, it is not yet possible to do this on a routine basis using human cells and eggs. One reason for lack of progress concerns the scarce supply of human oocytes available for research. Oocytes are usually obtained from women undergoing hormonal treatment for infertility, when permission is given for unused and unwanted oocytes to be used for research. However, a severe shortage and great demand for such material has stimulated efforts to recruit suitable donors from the general public, often with financial incentive. There is significant health and ethical concern about such policies and the potential negative impacts of such procedures on long-term health of women are unclear.

Remarkably, recent research with mice suggests that it may be possible to produce oocytes from hES cells. If so, the oocytes produced may be of use for SCNT to produce new lines of personalized-hES cells for treatment of patients. If successful, this would be expected to have at least two major benefits to the public. First, women would no longer be required as a source of eggs for research, which would reduce the risk of such treatments on women's long-term health. Second, this would generate a theoretically infinite increase in the quantity of oocytes available for research, which would in increase the rate at which technical advances could be made in production of immune-matched hES via SCNT.

Consequently, the proposed research will investigate methods for production of germ cells and oocytes from female hES cells. Specifically, we will test our prediction that it is possible to coax hES cells in culture to form germ cells and ultimately mature oocytes by exposing hES cells to different cell proteins and hormones that are normally used by the body to generate eggs. If successful, these protocols will help accelerate research on development of therapeutic cloning for a wide range of diseases. This would also obviate ethical concerns regarding egg-donation by women and would help protect women's health.

Induction of pluripotency in fibroblasts by fusion with enucleated human embryonic stem cell syncytia
University of California, San Francisco
Principal Investigator: Holger Willenbring, M.D. Leon J. Thal SEED Grant Program

Embryonic stem cells are pluripotent which means they can in principle be instructed to become every cell type in the body. Moreover, they can produce an infinite number of daughter cells. Therefore, human embryonic stem cells have great potential as a cell source for regenerative therapies of a wide range of diseases, some of which require the replacement of hundreds of millions of cells.

A major obstacle towards the realization of regenerative therapies using for example neurons or liver cells derived from human embryonic stem cells is the immune reaction they provoke after transplantation. This is caused by markers all differentiated cells display on their surface which enable the body's immune system to distinguish potentially harmful foreign structures from its own cells. These so called histocompatibility markers are encoded in the genome and differ significantly between most humans which necessitates suppression of the immune system before incompletely matched cells or organs can be transplanted. Drugs effective at long-term immune suppression can cause severe side effects. Therefore, the creation of pluripotent stem cells that are matched to the recipient's histocompatibility complex would be desirable.

Replacing an oocyte's nucleus with a nucleus from a fibroblast has been shown to lead to reprogramming and acquisition of pluripotency by the somatic cell's nucleus. This so called somatic cell nuclear transfer has highlighted an opportunity for the creation of pluripotent stem cells that would be perfectly matched for transplantation since they contain only the recipient's genome. However, both ethical and technical obstacles have hampered the development of this technology. In particular, the need for large numbers of oocytes has restricted this research to only a few laboratories in the world.

Recently, a process called cell fusion has been found to enable the use of human embryonic stem cells for reprogramming of somatic cells. Cell fusion describes the melding of two or more cells which produces a single cell encompassed by the parental cells' membranes and containing their nuclei and cytoplasms. To render these fusion products useful for transplantation, the genome of the human embryonic stem cells would have to be eliminated.

This can be achieved by centrifugation but appears to impede the reprogramming potential of embryonic stem cells. In contrast, oocytes retain reprogramming activity after enucleation which is attributed to the accumulation of nuclear factors in their large cytoplasm. The cytoplasm of human embryonic stem cells is small which we will compensate for by creating large embryonic stem cell fusion products. Based on our experience with mouse embryonic stem cells, these fusion products will retain pluri-potency and will undergo nuclear fusion. The resulting single, large nucleus can be completely removed and the increased availability of nuclear factors is expected to afford high reprogramming potential.

In Vivo Imaging of Human Embryonic Stem Cell Derivatives and Tumorigenicity

Stanford University
Principal Investigator: Joseph C. Wu, M.D., Ph.D. Leon J. Thal SEED Grant Program
Also referenced in: Genomic Instability and Cancer

Human embryonic stem cells (hESCs) are one of the most fascinating subjects of interest in all of biology and medicine these days. Under certain physiologic conditions, they can be induced to become specialized cells such as brain, cardiac, liver, pancreatic, and bone marrow cells. This opens up the exciting possibility that hESCs may one day be used to treat patients with Parkinson's disease, heart conditions, hepatitis, diabetes, and leukemia, just to name a few currently intractable diseases that affect millions of Americans alone. This field of cell-based therapies to treat human diseases is generally referred to as "regenerative medicine."

Scientists who want to study hESCs or their specialized cell derivatives typically inject them into small animal models such as mice and rodents. However, researchers currently are unable to monitor noninvasively these cells after transplant. Instead, these animals are typically sacrificed for postmortem biopsy, which precludes long-term follow-up of transplanted cells. Without the ability to follow the progress of transplanted cells over a longer period of time, important insights into hESC fates in vivo have not been forthcoming. Thus, developing a novel technology to track transplanted hESCs and their specialized cell derivatives would represent a major advancement in this field that will produce wide-ranging theoretical and practical implications.

Another problem with transplantation of hESCs is the potential to cause teratomas. Teratomas are disorganized arrays of cell differentiation that appear to recapitulate many of the events involved in early embryonic development. Clearly, the teratoma formation risk is a major obstacle to future clinical application of hESCs. In this proposal, we will evaluate how teratoma forms in living subjects over time using the imaging techniques that we have developed as well as how best to prevent them in the first place.

Due to the serious risks posed by teratoma formation, it is necessary to induce hESCs to become specialized cell derivatives first before transplantation for therapeutic purposes. However, this process is not efficient at present despite intense efforts searching for methods to expedite it. Our team plans to tackle this problem using the latest genomics and proteomics technology.

In summary, our proposal is a targeted response to the CIRM SEED grant. It seeks to develop a novel technology (molecular imaging) that will address a critical barrier in clinical translation of hESC therapy (teratoma formation) and provide a better understanding of cardiac cell differentiation process (genomics/proteomics). Our well-established multidisciplinary team has the required training, experience, and innovation to complete the project. Overall, we are confident that our proposed studies will generate significant progress in this field, in both scientific knowledge and useful therapies.

Functional Genomic Analysis of Chemically Defined Human Embryonic Stem Cells
Stanford University
Principal Investigator: Julie C. Baker, Ph.D. Comprehensive Research Grant Program

Regenerative medicine holds the promise that tissues can be engineered in vitro and then transplanted into patients to treat debilitating diseases. Human Embryonic Stem Cells differentiate into a wide array of adult tissue types and are thought to be the best hope for future regenerative therapies. This grant has three main goals: 1. The creation of new human embryonic stem cells in animal free conditions which will allow for future therapeutic uses. 2. The creation of human embryonic stem cell that contain mutations in their genomes that cause diseases, including cystic fibrosis, muscular dystrophy, Downs Syndrome and many others. These lines can be used to study these diseases and to test potential therapies 3. A close biological assessment of one of the first tissues to arise during differentiation of human embryonic stem cells the endoderm. Since the endoderm eventually, during many days of development, becomes the pancreas, liver, and gut. It is critically important that we know everything about this very specialized tissue if we are to attempt to engineer these organs in the laboratory. Our overwhelming goal is to provide tools that clinician can use to treat disease whether it is to establish new and improved human embryonic stem cell lines or to find new ways of creating endodermal tissues within the laboratory for future therapeutic uses.

Human oocyte development for genetic, pharmacological and reprogramming applications
Stanford University
Principal Investigator: Renee A. Reijo Pera, Ph.D. Comprehensive Research Grant Program

There is much excitement over the possibility of new and fundamentally-different therapeutic applications for human embryonic stem cells and the cells to which they differentiate. In addition, there is equally great excitement in the scientific community regarding the potential of human embryonic stem cells for biological studies of how different human cell types differentiate into normal and diseased tissues. To date, there are more than 400 human embryonic stem cell lines that have been reported to have been derived from human embryos that are donated for research due to lack of suitability for transfer for reproduction or being in excess of the needs for reproductive purposes. The methodology to derive new human embryonic stem cell lines was largely derived from years of work in the clinic that resulted in optimal conditions to grow human embryos and from years of work in animal species, mostly the mouse.

In spite of this great progress, we have not, however, yet derived chromosomally-normal (genetically-normal) human embryonic stem cell lines via reprogramming techniques such as somatic cell nuclear transfer. In part this is due to the inaccessibility of human oocytes or eggs for potential therapeutic and basic science applications. In this proposal, we seek to differentiate human oocytes/eggs from multiple hESC lines and use them to reprogram somatic cell DNA. Specifically, we will: 1) Assess and compare the ability of multiple nonfederal hESC lines to contribute to the germ cell lineage (form immature oocytes). 2) Differentiate hESCs to mature oocytes/eggs. 3) Assay the ability of oocytes derived in vitro, relative to immature oocytes donated for research, to reprogram a somatic nucleus. We expect to successfully complete this research with the end results contributing to the improvement of current assisted reproductive practices such as IVF, and improved ability to detect errors in oocytes that lead to birth defects. In addition, since oocytes are particularly susceptible to genetic and environmental perturbation, we expect to develop a system useful for others who study oocyte biology and potential impacts that may cause their demise. Finally, we hope to generate a novel cell source for reprogramming of human somatic cells. This research is clearly aimed at a central issue of women's health as the formation, maintenance and demise of oocytes significantly impacts female physiology and health. The research is not eligible for federal funding as it relies heavily upon hESC lines that are excluded from federal funding and involves somatic cell nuclear transfer.

The Dangers of Mitochondrial DNA Heteroplasmy in Stem Cells Created by Therapeutic Cloning

University of California, Irvine
Principal Investigator: Douglas C. Wallace, Ph.D. Comprehensive Research Grant Program
Also referenced in: Genomic Instability and Cancer

In therapeutic cloning, a patient's cell is combined (fused) to an enucleated donated egg (oocyte) from an unrelated woman or from another animal. It is hoped that cellular factors in the egg cytoplasm will reprogram the patient's cell nucleus making it capable of generating replacement cells for the patient's body. Thus, if a patient is suffering from Parkinson Disease due to loss of brain cells, these cells could be replaced with differentiated, individualized, nuclear-transplantation, embryonic stem (ntES) cells. While this strategy should generate ntES cells with the patient's nuclear DNA (nDNA), it overlooks the fact that another part of the cell, the mitochondrion, also has DNA, the mitochondrial DNA (mtDNA). While the nDNA contains the blueprints for assembling the structure of the cell and body, analogous to carpenter's plans for a house; the mtDNA contains the blueprints for the cellular electrical system, the wiring diagram of the house. It is universally agreed that mixing the nDNAs from two different cells would be destructive, yet the potentially disastrous effects of mixing different mtDNAs has been overlooked. In electricity, randomly mixing the components of two different integrated electrical circuits will result in short circuits. The same appears to be true for the cell. In mice in which we artificially mixed two mtDNAs, the resulting mice aged and died prematurely, had a striking increased frequency of cancer, and an increased mtDNA mutation rate. Moreover, in human studies, the accumulation of mtDNA mutations has been associated with aging and the development of cancer. Therefore, to document what happens to the mtDNAs during the creation and growth of htES and hES cells, we propose to create ntES cells by fusion of human cells to enucleated rabbit eggs and then to monitor the fate of the human and rabbit mtDNAs. We will also determine if the mitochondria and mtDNAs of hES cells can influence the differentiated state of tissue cells, investigate the nature and extent of mtDNA mutations that accumulate in ES cells, and determine if mixing different mtDNAs in cells is deleterious.Then we will determine the effects of these various mtDNA genetic factors on the power output of the mitochondria and its effects on the ES cell's capacity to differentiate and potentially to form tumors. Finally, we propose to develop a series of procedures to control the origin and nature of the mtDNAs in ntES and hES cells and to use changes in mitochondrial function to regulate ntES and hES cell growth and regulation. It is our hope that by utilizing mitochondrial biology and genetics it may be possible to develop strategies for creating individualized stem cells without using donated oocytes.

CHAPTER 3

SHARED RESEARCH LABORATORIES AND STEM CELL TECHNIQUES COURSE

Federal policies in effect since 2001 restrict the use of resources funded by the National Institutes of Health (NIH) to human embryonic stem cell lines approved by presidential policy. These policies prevent researchers from conducting studies on "unapproved" lines - human embryonic stem cell lines developed since August 9, 2001. As more and more new lines become available, "NIH-free" laboratory space and equipment has become a limiting factor in the scientific stem cell research community. In addition to these constraints, few researchers have the necessary ability and competence to derive, grow, maintain, freeze and analyze these cells since the field is new and expertise is scarce. The lack of space and know-how are major bottlenecks to expansion of the field. To address these needs, CIRM issued a Request for Applications (RFA 07-01) in January 2007 to support the expansion of laboratory space and to train investigators interested in using human embryonic stem cells in their research.

The Shared Research Laboratory component of RFA 07-01 provides funds for the renovation of laboratory space and purchase of equipment dedicated to human embryonic stem cell research at academic and not-for-profit organizations throughout the state. In June 2007, a total of 17 laboratories were approved for funding to establish CIRM Shared Research Laboratory facilities. Eleven of these laboratories are located at universities and six at research institutes that support CIRM-funded stem cell programs. In many cases, the CIRM-funded space will complement existing facilities that were originally established by the host institution in support of stem cell research. These Shared Research Laboratories will allow investigators from multiple institutions to conduct studies on a wide range of human embryonic stem cells, including lines not approved by the federal government. The CIRM funding includes three years of support for the operation of these laboratories to ensure that this important resource will be widely available to the community of human embryonic stem cell researchers throughout the state for a substantial period of time.

A second part of the RFA, the component on Stem Cell Techniques Courses, includes operating and capital funds for training scientists and technical staff in handling human embryonic stem cells. These courses, which include hands-on instruction in culture techniques, will be given several times a year at six locations throughout the state, and rely on shared use of the CIRM-funded laboratory space and equipment for conducting the courses. The awards for the Stem Cell Techniques Course include funds for additional space and equipment dedicated exclusively to support the techniques course where needed.

Of the total nearly $50 million approved for funding applications to RFA 07-01, approximately $15 million represents expenditures for capital improvements as specified in Proposition 71. The balance of $35 million for equipment and operating costs is funded under the research component as defined in Proposition 71.

SHARED RESEARCH LABORATORIES AND STEM CELL TECHNIQUES COURSE PROGRAM

North Bay CIRM Shared Research Laboratory for Stem Cells and Aging
P.D.: Xianmin Zeng, Ph.D.
Buck Institute for Age Research
$1,725,000 for 3 years
(includes Techniques Course)

Collaborative Labortory and training course for Human Embryonic Stem Cell Research at Burnham Institute for Medical Research
P.D.: Jeanne F. Loring, Ph.D.
Burnham Institute for Medical Research
$1,660,000 for 3 years
(includes Techniques Course)

The Childrens Hospital Los Angeles hESC Facility
P.D.: Gay Miriam Crooks, M.B.B.S.
Childrens Hospital of Los Angeles
$870,000 for 3 years

The Gladstone CIRM Shared Human Embryonic Stem Cell Core Laboratory
P.D.: Deepak Srivastava, M.D.
The J. David Gladstone Institutes
$810,000 for 3 years

Shared viral vector facility for genetic manipulation of human ES cell
P.D.: Inder M. Verma, Ph.D.
The Salk Institute for Biological Studies
$820,000 for 3 years

TSRI Center for hESC Research
P.D.: Peter Schultz, Ph.D.
Scripps Research Institute
$790,000 for 3 years

The Stanford University Center for Human Embryonic Stem Cell Research and Education
P.D.: Renee Ann Reijo Pera, Ph.D.
Stanford University
$1,700,000 for 3 years
(includes Techniques Course)

The Berkeley Human Embryonic Stem Cell Shared Research Laboratory
P.D.: David Vernon Schaffer, Ph.D.
University of California, Berkeley
$815,000 for 3 years

UC Davis Translational Human Embryonic Stem Cell Shared Research Facility
P.D.: Alice Tarantal, Ph.D.
University of California, Davis
$845,000 for 3 years

The University of California: Irvine Regional Human Embryonic Stem Cell Shared Research Laboratory and Stem Cell Techniques Course
P.D.: Peter J. Donovan, Ph.D.
University of California, Irvine
$1,580,000 for 3 years
(includes Techniques Course)

CIRM Shared Research Laboratories
P.D.: Owen N. Witte, M.D.
University of California, Los Angeles
$860,000 for 3 years

Dollar amount shown is the amount approved by the ICOC rounded to the nearest $5,000, not the amount awarded.

A Stem Cell Core Facility for Studying Human Embryonic Stem Cell Differentiation
P.D.: Prudence Talbot, Ph.D.
University of California, Riverside
$815,000 for 3 years

Enhancing Facilities for Genetic Manipulation and Engineering of Human Embryonic Stem Cells at UCSD
P.D.: Karl Heinrich Willert, Ph.D.
University of California, San Diego
$850,000 for 3 years

The University of California San Francisco Shared Research and Teaching Laboratory: a Non-Federal Human Embryonic Stem Cell Resource for the Bay Area Community
P.D.: Linda C. Giudice, M.D., Ph.D., MSc
University of California, San Francisco
$1,590,000 for 3 years
(including Techniques Course)

UCSB Laboratory for Stem Cell Biology and Engineering
P.D.: Dennis O. Clegg, Ph.D.
University of California, Santa Barbara
$795,000 for 3 years

UCSC Shared Stem Cell Facility
P.D.: Lindsay Hinck, Ph.D.
University of California, Santa Cruz
$855,000 for 3 years

USC Center for Stem Cell and Regenerative Medicine: Shared Research Laboratory and Course in Current Protocols in Human Embryonic Stem Cell Research
P.D.: Martin F. Pera, Ph.D.
University of Southern California
$1,685,000 for 3 years
(includes Techniques Course)

Dollar amount shown is the amount approved by the ICOC rounded to the nearest $5,000, not the amount awarded.

ABSTRACTS

[Provided by applicant]

North Bay CIRM Shared Research Laboratory for Stem Cells and Aging

Buck Institute for Age Research Program Director: Xianmin Zeng, Ph.D.

Age-related diseases of the nervous system are major challenges for biomedicine in the 21st century. These disorders, which include Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis and stroke, cause loss of neural tissue and functional impairment. Currently, there is no cure for these devastating neurological disorders. A promising approach to the treatment of age-related neurological disorders is cell therapy, i.e., transplantation of nerve cells into the brain or spinal cord to replace lost cells and restore function. Work in this field has been limited however, due to the limited availability of cells for transplantation. For example, cells from 6-10 human fetuses obtained 6-10 weeks post-conception are required for one patient with Parkinson's disease to undergo transplantation.

Human embryonic stem cells (hESCs) offer a potentially unlimited source of any cell type that may be required for cell replacement therapy, due to their remarkable ability to self-renew (they can divide indefinitely in culture) and to develop into any cell type in the body. In this proposal, we will build out approximately 3400 square feet of shared laboratory space within our existing research facility for hESC research, as well as approximately 2400 square feet for classroom facilities dedicated to training in hESC culture and manipulation. We seek to understand how hESCs differentiate into authentic, clinically useful nerve cells and will use novel molecular tools to examine the behavior of cells transplanted in animal models of human neurological disease. We will also need to develop a noninvasive method of following cells after transplantation and we propose to develop luciferase-tagged (light-emitting) hESC lines for in vivo animal imaging. In addition, we will use hESC-derived nerve cells to screen drug and chemical libraries for compounds that protect nerve cells from toxicity, and to develop in vitro disease models. We believe that these experiments are critical to enhancing our understanding of neurological diseases and providing the tools that will be necessary to move cell therapy to the clinic.

Before a hESC-based therapy can be developed, it is essential to train scientists to efficiently grow, maintain and manipulate these cells. We propose to teach four 5-day hands-on training courses - two basic and two advanced hESC culture courses per year - to California scientists free of charge. These courses will provide scientists with an understanding of hESC biology and will enable them to set up and conduct hESC research after completion of training.

In summary, the goal of this proposal is to provide over twenty investigators at the home institute and neighboring institutions with the ability to culture, differentiate, and genetically manipulate hESCs - including clinical-grade hESC lines - to develop diagnostic and therapeutic tools.

Collaborative Labortory and training course for Human Embryonic Stem Cell Research at Burnham Institute for Medical Research

Burnham Institute for Medical Research Program Director: Jeanne F. Loring, Ph.D.

We are proposing to expand our "safe haven" human embryonic stem cell laboratory to accommodate the enormous interest in scientific research in this field, and to provide an environment that is conducive to the goals of the CIRM's Strategic Plan. Our collaborative Shared Laboratory will support the research of all of our institution's many stem cell researchers, including the new investigators who have been recently approved for funding under the CIRM's SEED grant program. In addition, we will cooperate will neighboring institutions to minimize overlap in strategic technological areas and maximize the value of

CIRM's investment in our scientific community. The scientists in our program will share their special expertise in the areas of human ES cell derivation and molecular analysis.

All aspects of the Shared Laboratory will be directed by the Program Director, a well-established senior stem cell scientist who has experience in laboratory design and management of large groups of researchers. An Oversight Committee, composed of leading scientists, ethicists, and institution management will meet regularly to monitor and oversee the activities of the Laboratory.

We will also offer a series of Basic and Advanced Stem Cell Techniques Courses on behalf of our local scientific community. A Public Education Program will provide non-scientists with the opportunity to have hands-on experience with hESC research. Alumni from the courses will have access to an interactive web-based discussion group, and will meet once a year to share their scientific discoveries and insights. By closely collaborating with other California institutions, we plan to take full advantage of CIRM's investment in stem cell research and speed the translation of stem cell-based therapies to the clinic.

The Childrens Hospital Los Angeles hESC Facility

Childrens Hospital of Los Angeles Program Director: Gay Miriam Crooks, M.B.B.S.

Our institution is a tertiary-care academic pediatric medical center that combines care of severely ill children, research into the causes and treatments of childhood disorders, and training of the next generation of pediatric clinical physicians, nurses and allied health care professionals and biomedical scientists. A unique focus of the research in our institution is on applications to pediatric disorders such as diabetes, inherited disorders (cystic fibrosis, muscular dystrophy, sickle cell disease, etc), cancer and congenital birth defects. It is our central hypothesis that childhood disorders will be especially responsive to therapies produced by the use of stem cells; advances in the use of stem cells to treat childhood illnesses will then lead the way to treatments for the many disorders that occur later in life. For over a decade, the Stem Cell Program at our institution has been at the leading edge of translational research for cell and gene therapy and tissue engineering, with outstanding research programs in stem cells, gene therapy, developmental biology, organogenesis and transplantation immunology. Active research programs studying adult stem cells (hematopoietic, mesenchymal, pancreatic, hepatic, pulmonary, amniotic) and human and murine embryonic stem cells, interact closely with clinical Centers of Excellence in organ and hematopoietic stem cell transplantation, diabetes, cancer and blood diseases, neonatology, as well as a full array of pediatric secondary and tertiary care programs. These academically-oriented clinical programs have a long-standing tradition of inter-weaving research and clinical trials with patient care, to develop and evaluate innovative new treatments for severe pediatric and adult disorders. A Core Laboratory for studies with human embryonic stem cells (hESC) was established in 2005, using institutional funding. The hESC Core has supported initial studies and developed a formal training program in methods for the growth of hESC; 40 scientists from 5 research institutions in Southern California have attended the training course to date. However, the technical and regulatory burdens inherent in hESC research, have significantly restricted further development of individual hESC research projects within the limited existing laboratory space at our institution. Funding is thus requested to remodel and equip approximately 3000 sq ft of existing space (2500 sq ft of usable laboratory space) to create a suite of laboratories for dedicated use as an hESC Core facility alongside shared laboratory space for investigators involved in hESC research. We anticipate the laboratories and equipment established using this grant will support the research of at least 20 scientist investigators at our institution and will be also made available to researchers at nearby institutions across Southern California.

The Gladstone CIRM Shared Human Embryonic Stem Cell Core Laboratory
The J. David Gladstone Institutes Program Director: Deepak Srivastava, M.D.

The CIRM Shared Human Embryonic Stem Cell Core Laboratory will provide shared research facilities for use by California scientists. This laboratory will be hosted by a research institution focused on basic research into three of the most important medical problems of modern times: cardiovascular disease, AIDS, and neurodegenerative disorders. Each of these research areas addresses promising targets for regenerative medicine. We propose to develop a laboratory (1108 sq ft) for hESC tissue culture with specialized microscopy, and an animal holding and procedure space (500 sq ft) for in vivo pre-clinical studies of hESCs in mouse models of disease. The proposed laboratory will also help to train students from a nearby college be become laboratory technicians. This facility will contain advanced equipment for analyses of hESCs and complement existing space and incorporate hESC work provided by other core laboratories such as the genomics and flow cytometry cores that serve a broad community of researchers.

The host institution is renowned for the quality and administration of its extensive core facilities. Highly productive cores have always been at the heart of this institution's culture and this continues to be a priority. Five years ago, the host institution founded an embryonic stem cell core, which allows investigators not familiar with ESC research to obtain training, expertise and knowledge regarding embryoid bodies and ESC differentiation. As a result, two-thirds of the current investigators have incorporated some aspect of stem cell research in their portfolio. The host institution is also located in close proximity to a major biomedical university, so that all stem cell services are being coordinated to provide the best possible array of services to all stem cell investigators.

The research interests of our investigators that are related to stem cells can be grouped into three areas: cardiovascular development and disease, neurodegeneration and repair, and mechanisms that control the genetic stability of the cells while they divide and develop. This research involves the creation of genetically altered ESCs that require maintenance, expansion, and characterization. To aid in the analysis of the cellular phenotypes, we propose to use advanced high-content microscopy equipment. Several leading laboratories that apply this technology to basic cell biological analysis are close to Gladstone. An important next step will be to examine the behavior, survival, and interactions of hESCs once they have been implanted into mice. Visualization of the cells in live animals will be greatly enhanced by the proposed imagining instrument that will allow us to examine living cells within animals by light signals transmitted from the implanted cells. This program represents a comprehensive basic approach to how stem cells develop into other kinds of cells and will form the foundation for future preclinical studies.

Shared viral vector facility for genetic manipulation of human ES cell
The Salk Institute for Biological Studies Program Director: Inder M. Verma, Ph.D.

Human ES (hES) cells offer the opportunity to be converted into replacement tissues for diseased organs and provide cures for diseases like Parkinson's, diabetes, and a host of neurological disorders. Unfortunately due to political considerations, scientific space containing equipment and other resources provided by the federal government are off limits for work on unapproved hES cell lines. Space where unimpeded hES cell work can be carried out is a major limitation for many scientists at our institute to initiate scientific inquiry in the very exciting field of regenerative medicine. We are applying for funds for a Shared Research Laboratory Grant to work on non-federal government-approved hES cells, which are superior to the approved hES cell lines. Eighteen faculty here have no laboratory space in which to do research in hES cells. We are requesting funds to renovate and equip ~2,000 sq ft of independently-operated, stand-alone space which will include laboratory space for generating lentiviral vectors (LV) capable of delivering genes and/or small interfering RNA (siRNA) to manipulate hES cells, and tissue

culture facilities to grow and propagate non-NIH approved hES cell lines. To study and to induce differentiation of hES cells to different lineages we will require the availability of tools that activate or suppress gene expression. Furthermore to correct a genetic defect, for instance a defective insulin gene that leads to type I diabetes, one needs to introduce a functional insulin gene in the regenerated pancreas from the ES cells of a diabetic patient. If the introduced gene is not integrated in the genome, it will be lost in the subsequent progeny. Thus the need for a delivery vehicle that will become part and parcel of the chromosome in both the progeny cells and the self-renewing cells. We have developed delivery vehicles based on retro- and lentiviruses that can safely and efficiently deliver and transcribe genes in both the embryonic and adult stem cells. We believe that we can provide such vectors to all the stem cell researchers in the neighboring institutions working on CIRM related hES cell projects, which will not only be cost effective, but accelerate the pace of science in the exciting field of hES cells. We plan to hire a full-time core director and 3 research assistants who will be responsible for setting up and maintaining the stem cell facility and managing the core facility for generating viral vector.

TSRI Center for hESC Research
Scripps Research Institute Program Director: Peter Schultz, Ph.D.

The therapeutic use of stem cells in regenerative medicine will require the ability to control stem cell expansion and differentiation into specific tissue types, such as pancreatic β-cells, heart tissue, bone or specific neuronal lineages. We have taken a chemical approach toward this problem in which large collections of synthetic small molecules are being screened in cell-based assays to identify drug-like molecules that control stem cell processes. Preliminary experiments in our institute have demonstrated that we can identify molecules that control the self-renewal and directed differentiation of murine embryonic stem cells. The characterization of the biological mechanisms of the molecules has also provided new insights into the underlying biology of stem cells. We now propose to extend these studies to hESC lines not eligible for federal funding, for which our research activities have been restricted to date. In addition, such lines may be better suited for specific applications, including the use of small molecules to derive specific cell lineages and investigate ES derived cell-based models of genetic disease. To this end, we would like to establish a human embryonic stem cell core facility. This facility will house the necessary equipment to genetically manipulate and culture hESCs on a large scale for a variety of studies including cell-based screens of small molecule libraries, as well as screens of arrayed genomic cDNA and siRNA libraries. We anticipate that this facility will serve our faculty as well as other labs that would like to collaboratively exploit this chemical approach to the study and manipulation of stem cells.

The Stanford University Center for Human Embryonic Stem Cell Research and Education
Stanford University Program Director: Renee Ann Reijo Pera, Ph.D.

The goal of this proposal is to establish a premiere center for human embryonic stem cell (hESC) research and education in the state of California. Our center builds on the established excellence of faculty with research organized into four thematic areas: 1) Human embryology, derivation of hESC lines, including disease-specific lines, and SCNT, 2) Cell fate specification and hESC reprogramming, 3) Cancer and cancer stem cells, and 4) Directed differentiation to cardiac and neural lineages..

Here, we seek funding to renovate facilities that will house a human embryo/oocyte resource center and database, hESC line derivation, as well as other research and educational training including a central repository for growth, characterization and distribution of hESC lines to scientists in our community. The success of the faculty in this Center in garnering funding for hESC research, including CIRM funding, mandates the expansion of our research facilities. In addition, an accompanying curriculum in Stem Cell Techniques Courses is complementary to the research efforts and builds on a history of teaching

excellence. This curriculum will encompass three areas: 1) Basic hESC Biology covering core essentials of hESC biology for individuals with little or no previous experience in hESC research, 2) Advanced or Specialized Stem Cell Techniques courses that will provide individuals with tailored instruction to enhance forward momentum in selected scientific topics, and 3) Systems Biology that reaches across institutions to bring together scientists in hESC and computational research. We anticipate that the outcome of our training initiatives will be both an expansion of knowledge and the building of teams to tackle tough basic and clinical challenges.

Finally, we note that our human embryo/oocyte resource center will provide expertise, materials and a complete, decoded database for use of precious resources in hESC research. This will enhance efforts to provide early diagnostics for reproductive and somatic disorders, cancers and onset of disease. Thus, this Center builds on a regionally unique combination of scientific and clinical excellence of Stanford University and neighboring institutions to provide critical research and educational support to scientists in California.

The Berkeley Human Embryonic Stem Cell Shared Research Laboratory
University of California, Berkeley Program Director: David Vernon Schaffer, Ph.D.

Investigators from three major regional research and clinical institutions have instituted a stem cell research center. Numerous collaborations among our community of investigators have successfully utilized both Federal registry and non-registry human embryonic stem cell (hESC) lines in the center; however, the available resources for the culture and maintenance of these lines place inherent limitations on the research. We therefore propose to establish a Human Embryonic Stem Cell (hESC) Shared Research Laboratory for cell culture and investigation, which will serve as a central resource to greatly enhance stem cell science and technology in the region.

This resource will greatly benefit numerous ongoing research project areas. First, the ability of human embryonic stem cells to self-renew, that is grow and maintain their ability to differentiate into presumably every cell type in the adult body, is a hallmark property this is incompletely understood. Investigations of self-renewal mechanisms will lead to improved approaches to mass produce these cells for numerous therapeutic and diagnostic applications. In addition, understanding how hESCs differentiate into blood cells will enhance the treatment of numerous disorders including cancer, diabetes, and infectious disease. Moreover, studying how hESCs differentiate into numerous types of neurons will have implications for neurodegenerative disorders, including Parkinson's Disease and Lou Gehrig's Disease. Furthermore, regenerative medicine efforts to engineer new cardiomyocytes and blood vessels will improve the treatment of heart disease and congestive heart failure, still the leading loss of life in the United States. Importantly, the ability to control and harness hESCs as a limitless source of differentiated blood cells, neurons, cardiomyocytes, and other cell types will also greatly enhance high throughput drug screening, toxicology screens, and diagnostics efforts. Finally, novel bioengineering approaches to create robust and scaleable technology platforms for expanding, differentiating, and grafting hESCs will benefit all such therapeutic and diagnostic applications.

The Shared Research Laboratory will be located in two nearby sites on the host institution, within a new building and a modern biological sciences building, to provide convenient access to all researchers on campus and in the surrounding community. The Laboratory will provide a central repository and resource for culture and maintenance of numerous lines, and imaging and cytometry analysis. The Laboratory will also build upon our strong tradition of and success with shared core facilities to synergistically enhance our stem cell research capabilities. The resulting culture and analytical facility will thus provide a strong, shared resource to benefit stem cell research in the regional community.

UC Davis Translational Human Embryonic Stem Cell Shared Research Facility
University of California, Davis Program Director: Alice Tarantal, Ph.D.

The intent of the proposed shared research facility is to provide a state-wide resource for qualified scientists in California to study human embryonic stem cells (hESC) without federal restrictions. The shared facility will encourage a spirit of collaboration and include laboratories for investigators to culture, collect, store, and analyze hESC, provide necessary services that will be costeffective and assist with research productivity, and ensure an environment that will facilitate the essential interactions among scientists. This approach will advance the use of hESC for regenerative medicine purposes and aid in developing new technologies and therapies for the treatment of human disease. Using established methods that have proven successful for other collaborative and service-based structures, this facility will encourage scientists to work together and provide the necessary resources to ensure their success. Investigators new to hESC research will benefit greatly by having this facility available because it will have a centralized supportive structure where experienced personnel will provide the necessary assistance and guidance. For those investigators with hESC research experience, new opportunities will be available to work with cell lines that can be obtained but not be used in laboratories that are supported by federal funding. This will greatly expand research programs that are focused, for example, on studying ways to differentiate hESC towards blood cells and vessels for the treatment of disorders such as sickle cell disease and vascular abnormalities associated with heart disease. In addition, regeneration of damaged organs such as the heart, lung, liver, or kidney may require methods to reconstruct these tissues using scaffolds on which to grow the cells. These approaches require the ideas of cell biologists, engineers, biomedical researchers, and clinicians working together, and testing these ideas to ensure the procedures are safe before considering treatments of human patients. Techniques such as those that focus on ways to monitor cells once they are injected into the body will provide a powerful tool to study the outcome of these therapies.

The University of California: Irvine Regional Human Embryonic Stem Cell Shared Research Laboratory and Stem Cell Techniques Course
University of California, Irvine Program Director: Peter J. Donovan, Ph.D.

A major goal of the Shared Research Laboratory (SRL) is to foster the development of new treatments for human diseases and disorders by serving as a leading regional center for human embryonic stem cell (hESC) research, clinical applications, and training. A critical component of this vision is a full service SRL. The SRL will provide space and equipment that is free of federal funding to allow pursuit of any study needed to discover the basic properties of hESCs, to understand disease processes, to accelerate drug development and to develop cell-based therapeutics. The research in the SRL includes a balance of studies into the basic biology of hESCs, disease mechanisms, and potential therapeutics. Results of these studies will increase our understanding of the causes and potential treatments of spinal cord injury, retinal disease, motoneuron diseases, Huntington's disease, diabetes, multiple sclerosis, muscular dystrophy, heart disease, and Alzheimer's disease. The SRL also hosts a hESC Techniques Course. This 5-day, intensive, hands-on course trains future stem cell researchers in techniques for cultivation, handling and differentiation of hESCs. We propose to develop new space for pre-clinical testing, to obtain key pieces of major equipment, and to support personnel in order to improve our ability to develop new FDA-compliant treatments for human diseases and disorders. The new space will allow us to expand our training effort to include procedures needed to conduct pre-clinical translational and transplantation projects. The expanded curriculum will include animal survival surgery, cell transplantation techniques, and methods for tracing transplanted cells in the animal. Currently few, if any, venues exist in which researchers can learn not only how to create potential hESC therapeutics, but also learn how to test potential treatments in animal models. Importantly, all treatment-oriented research will be done under strict FDA quality assurance guidelines, so researchers will not have to repeat experiments when they file with the FDA, streamlining processes and decreasing time to clinical trial. The research expertise and

institutional support for hESCs puts us in a strong position to serve as a regional facility of excellence, bringing new researchers into the field, and leading the way toward realizing the potential of hESCs in treating human conditions.

Our institution is exceptionally strong in translating basic scientific discoveries to the clinic,and in particular, has FDA-compliant pre-clinical strength in translation of hESC discoveries. Indeed, preclinical studies undertaken through the SRL will be conducted under the guidance of existing Regulatory Quality Assurance Officers to ensure FDA-compliance. With the proposed additions to the SRL, our vision of serving as a regional resource for hESC research and training will bring us closer to hESC-based treatments.

CIRM Shared Research Laboratories
University of California, Los Angeles Program Director: Owen N. Witte, M.D.

Our plan is to establish a ~4700 sq. ft. shared research laboratory dedicated to the experimental manipulation and ultimate clinical application of human embryonic stem cells (hESC). This Shared Research Laboratory (SRL) is centrally located on the main campus. The SRL will be used by researchers focused on understanding how hESCs are induced to generate specialized tissues used for regeneration of the blood forming, nervous, and musculoskeletal systems. The SRL will be a state of the art facility accommodating a hierarchy of functions that includes:

~1659ASF of general hESC, multi-user laboratory space will be assigned on a time share basis to investigators who do not have the capacity, or cannot due to federal restrictions, conduct research with hESC in their own research laboratory. In addition to cell culture facilities that will allow multiple groups to work simultaneously, space in this area includes an hESC analytic laboratory for the basic characterization of hESC and their derivatives.

~2245ASF of space will be used to establish a hESC GTP suite in which hESC free of infectious agents can be experimentally manipulated in a manner commensurate with their future clinical use. In addition to equipment necessary for the growth and genetic manipulation of hESC under GTP conditions, this facility will be able to distribute GTP maintained hESC lines to investigators.

Adjacent to the hESC GTP suite is the GMP laboratory suite including a hESC GMP derivation laboratory and bank. These facilities will allow hESCs to be derived and their progeny manipulated under conditions that meet federal guidelines for patient use. We have a strong track record of applying basic research findings to patients, and the adjacent location of multi-user, GTP, and GMP laboratories is an important factor that will allow basic hESC research findings to be developed and used to treat various human diseases.

The space for the SRL is part of our commitment to hESC research that includes 12 new stem cell faculty positions and matching funds for laboratory development. A committee comprised of faculty with extensive experience in the growth and manipulation of hESC is currently planning the development of the Shared Research Laboratory, and once it is established, they will provide regulatory oversight and supervise three staff responsible for the quality control of all equipment, ordering supplies, and scheduling access. The CIRM Shared Research Laboratory will be a state of the art facility in which intra- and extra-mural investigators can conduct hESC research not allowed due to federal restrictions or not technically feasible in their own laboratories.

A Stem Cell Core Facility for Studying Human Embryonic Stem Cell Differentiation
University of California, Riverside Program Director: Prudence Talbot, Ph.D.

This application proposes to develop a Stem Cell Core Facility of ~1700 square feet to support the use of human embryonic stem cells (hESC) for a growing consortium of stem cell scientists at the home institution as well as neighboring institutions. The facility will be built and managed so as to allow use of non-NIH-approved hESC cell lines as well as research funded by nonfederal agencies including the California Institute for Regenerative Medicine (CIRM). The Facility will be centrally located adjacent to other existing, successful core facilities and within short walking distance of all the users at the home institution. The Facility will be managed by an Oversight Committee consisting of faculty experienced in hESC and associated technologies, as well as those with experience in managing shared core facilities. The Committee will have close contact with an established Biotechnology Impacts Center to address any ethical issues that may arise.

The users at the home institution consist of an energetic, interdisciplinary group of both young and established investigators who have made a substantial commitment to stem cell biology. Within the past several years, they have held workshops on embryonic stem cells with neighboring institutions, taught two graduate level courses in stem cell biology, including one in bioethics, established a Stem Cell Center, and applied for and received CIRM funding. They have recently hired an experienced hESC investigator and are currently recruiting others, demonstrating the home institution's commitment to the field of hESC. The group currently consists of 30 investigators from three different colleges within the home institution who have common interests in molecular mechanisms of pluripotency and differentiation of hESC.

Several investigators have joint projects, including collaborations with investigators at neighboring institutions who will also be using the facility. The proposed Stem Cell Core Facility will allow this dynamic group of accomplished investigators to bring the promise of stem cell biology to an expanding, culturally diverse region of California.

The research programs that would use the facility concentrate on various aspects of the molecular mechanisms underpinning the pluripotency of hESC, as well as their ability to differentiate into different types of tissues. The results generated by these programs will contribute to the development of tools, diagnostics, and therapies by laying the foundation for understanding hESC and identifying new compounds and methodologies that will allow researchers to maintain hESC and prepare them for use in therapies. This basic understanding of the molecular networks governing hESC biology is essential before any safe and effective treatment can be considered for use in humans.

Enhancing Facilities for Genetic Manipulation and Engineering of Human Embryonic Stem Cells at UCSD
University of California, San Diego Program Director: Karl Heinrich Willert, Ph.D.

Human embryonic stem cell (hESC) research promises to be of fundamental importance in the study and treatment of various human diseases, including cancer, neurodegenerative disorders and organ failure. In recent years we have made great strides in advancing hESC research as documented by the large number of successful, high-impact laboratories and breadth of research projects here. In addition, we are situated among several other first-rate institutions, all of which have joined in an unparalleled research environment for hESC research.

Since the creation of the California Institute for Regenerative Medicine, we have devoted both space and financial resources to promote hESC research. Our institutional commitment has as a cornerstone the creation of a core facility for hESC research to foster and promote hESC research at this and surrounding

institutions. To date the facility has served to (1) train scientists in the basic methodologies to conduct hESC research (2) facilitate hESC research for many investigators, both established and beginning scientists, and (3) provide a 'safe haven' that is sheltered from any federal funding sources thus allowing unimpeded hESC research. However, due to the high demand on space, equipment and technologies, the present facilities are insufficient to sustain the ongoing and proposed research projects.

We therefore request funding from CIRM to expand this facility and enhance its scientific output and creativity. In addition to providing expanded adequate facilities for our many scientists and clinicians embarking on hESC research, our major scientific goals for the shared research laboratory are (1) the development of protocols for the generation of genetically marked HESC lines, (2) the improvement of protocols for derivation of mature cell types, with an emphasis on neural differentiation, and (3) the development of novel surfaces and materials for the large scale growth and production of hESCs. These goals synergize the expertise of several departments, including the departments of Bioengineering, Materials Science, Biological Sciences, Pharmaceutical Sciences and the School of Medicine.

The support provided by this shared research grant will allow our institution to enhance our interdisciplinary stem cell program so that we may accelerate our goals of improving health and conquering diseases through regenerative medicine.

The University of California San Francisco Shared Research and Teaching Laboratory: a Non-Federal Human Embryonic Stem Cell Resource for the Bay Area Community

University of California, San Francisco Program Director: Linda C. Giudice, M.D., Ph.D.

The University of California, San Francisco (UCSF) has a long history of making innovative discoveries that change the way scientists and clinicians think about disease processes and their approaches to finding cures. Accordingly, researchers at this institution were quick to appreciate the enormous promise of human embryonic stem cells (hESCs) as research tools for understanding how the body normally works, thus laying the groundwork to identify disease-related aberrations. Therefore, in 2001, when the federal government decided to limit government funding to work with existing hESCs, which they banked, U.S. scientists were faced with a dilemma. Would we abide by these unprecedented restrictions, which meant that research would be limited to first-generation cells, or could we find ways to develop second-generation, higher-quality hESCs? Investigators on our applicant team took both approaches. Since UCSF contributed two hESC lines to the federal registry, our team members participated in the government's program to distribute these cells, which entailed teaching scientists how to use them. We also sought nonfederal funding sources to derive new hESC lines. Thus, we have a great deal of experience that is directly relevant to achieving the California Institute for Regenerative Medicine's (CIRM's) goal of establishing Shared Research Laboratories that also offer hands-on courses. We give the highest priority to teaching hESC techniques in the context of the ethical issues surrounding this work.

Here, we propose to expand the nonfederal laboratory space that already exists at UCSF. Renovating and equipping an adjacent lab will significantly increase our capacity for growing and analyzing second and subsequent generations of hESCs. Our goal is to make the existing space, renovated with UCSF funds, and the new lab to be created with this CIRM award, available to our colleagues. We also want to jump-start their work by teaching them how to grow and analyze hESCs. Thus far, 16 graduate- and postgraduate trainees are funded by our CIRM training grant; 32 UCSF scientists have applied for CIRM SEED and Comprehensive grants, and we expect many more will follow. We also want to support the work of our colleagues at 10 neighboring institutions. At the same time, we will use this lab to derive new and higher-quality hESC lines. We will also teach these techniques to highly motivated California scientists. Our work is important because the researchers who use our laboratory are studying the causes of major human diseases that occur as the result of trauma (e.g., paralysis), cell death (e.g., Parkinson's and Alzheimer's diseases, diabetes, cardiac failure), or cell malfunction (e.g., cancer). Thus, by sharing our laboratory space, scientific equipment and technical expertise with colleagues at UCSF and other

institutions, we will play an important role in helping scientists accomplish CIRM's ultimate goal of finding cures for human diseases.

UCSB Laboratory for Stem Cell Biology and Engineering
University of California, Santa Barbara Program Director: Dennis O. Clegg, Ph.D.

Regenerative medicine is an emerging area that will only realize its great potential through novel collaborative research approaches, and the University of California at Santa Barbara (UCSB) is well positioned to make significant contributions by leveraging fundamental biomedical research efforts with enabling technologies in materials, microfluidics and bioengineering. This proposal details plans for the development and renovation of shared-use laboratory facilities for the culture of human embryonic stem cells (hESC). The Laboratory for Stem Cell Biology and Engineering will be designed to promote stem cell research by investigators at UCSB, as well as those at neighboring universities and research institutions on the California central coast. Availability of a core stem cell laboratory will facilitate expansion of current stem cell studies at UCSB and stimulate new investigations into the biology and engineering of stem cells. The Laboratory will be embedded within a new UCSB Center for Stem Cell Biology and Engineering that is planned for the 3rd and 4th floors of Biological Sciences 2 building. Our clientele will include researchers in 13 different Departments and Institutes at UCSB, as well as nearby institutions. Research supported by the facility will include: investigations of the molecular mechanisms of hESC proliferation and differentiation; translational bioengineering to study novel methods of hESC culture, sorting, and delivery; and studies in regenerative medicine that test hESC derivatives in animal models of disease.

UCSC Shared Stem Cell Facility
University of California, Santa Cruz Program Director: Lindsay Hinck, Ph.D.

We have assembled a team of researchers with the aim of elucidating the molecular and cellular mechanisms that regulate stem cell self renewal and differentiation. Drawing on their broad range of expertise in development, genetics, genomics, molecular, cell and computational biology, these researchers will use interdisciplinary approaches to tackle problems concerning how genes are regulated in human embryonic stem cells (hESCs), and how this regulation influences their ability to both self-renew and differentiate into specific cellular subtypes. Defining and ultimately controlling this process is an essential step in designing stem cell-based therapies. These projects are aimed at providing insights and tools for neurological and genetic conditions such as Parkinson’s Disease, ALS, CHARGE Syndrome, and Down Syndrome, and in aiding the development of gene therapy strategies. The work is funded in part from CIRM SEED grants to our faculty. In addition, we are committed to campus growth in this area, with faculty hires slated for expertise in various aspects of stem cell biology. Supported by a CIRM Training Grant, we are also committed to training a new generation of stem cell researchers – graduate students and postdoctoral fellows who will gain the knowledge and skills to embark on their own careers in this field.

To achieve these goals, we propose to build a Shared Stem Cell Facility (SSCF) by renovating 2000 square feet of space in the building where hESC research currently occurs. Our institution currently has no stem cell facility - hESC research is currently limited to NIH-approved lines because of the lack of separate, appropriately funded space. In addition, this facility will significantly expand and enhance the research space available for experimentation with hESC, in general, at our institution. The creation of a central facility dedicated to hESCs is essential for both on-going and new research, as well as for training. The resources and expertise provided by the SSCF will encourage additional faculty to use hESCs in their research and create new opportunities for faculty already committed to hESC research. For example, our faculty are eager to initiate projects that involve the use of non-approved cell lines that are free of the biological limitations of the approved lines, such as new hESC lines in which the mechanisms of self renewal and differentiation are altered, and in lines bearing disease causing mutations. This work

will not be possible without a facility dedicated to hESC research that is free of federally-imposed restrictions.

USC Center for Stem Cell and Regenerative Medicine: Shared Research Laboratory and Course in Current Protocols in Human Embryonic Stem Cell Research

University of Southern California Program Director: Martin F. Pera, Ph.D.

To realize the potential of human embryonic stem cells (hESC) in research and medicine, it is essential to disseminate state of the art technology in this field to the scientific community at large. The Shared Research Laboratory (SRL) of the Center for Stem Cell and Regenerative Medicine (CSCRM) at the University of Southern California will aim to provide a comprehensive support service for hESC researchers at our University and at neighboring institutions. The mission of the SRL will include the following goals: 1) to supply scientists with quality controlled stem cell lines for use in their research, including cell lines that are not eligible for use in NIH-funded projects; 2) to provide space and equipment for scientists new to the field to carry out pilot projects, in order to help them to integrate the hESC platform technology into their own research programs; 3) to develop and validate new and improved methods for growing hESC in the laboratory; 4) to operate a formal practical course in hESC laboratory techniques to scientists from throughout the region.

The facility will be situated in the new Harlyne Norris Cancer Center tower on the USC medical school campus. The laboratory will have sufficient work stations to support training, collaborative projects, and research and development programs for evaluation of new stem cell culture techniques, and it will be equipped with specialized instruments required to monitor stem cells. The operation of the facility will be overseen by the Program Director and the Manager of the CSCRM Core Facility. Advice on access and management will be provided by a subgroup of the CSCRM Stem Cell Advisory Group comprising stem cell researchers from USC, Childrens Hospital of Los Angeles, and California Institute of Technology.

The SRL will support the work of CSCRM scientists and their colleagues at neighboring institutions involved in basic research on hESC, including international collaborations on standards for this research. The facility will also enable many groups involved in translational work at the USC medical school to gain experience and training in the use of hESC in their work in areas such as neurology, liver disease, cardiology, and ophthalmology. These scientists will be able to conduct preliminary studies in the facility under the guidance of experienced staff.

The SRL will offer a 5-day course on Current Protocols in Human Embryonic Stem Cell Research, to provide a comprehensive practical training for investigators wishing to use hESC lines in their research programs. Laboratory instruction will include demonstration of the most commonly used methods for cultivating hESC, methodology for assessing the purity and quality of hESC cultures, and methods for converting hESC into specific cell types such as nerve or blood cells. The training course will be available to scientists from institutions throughout the Los Angeles area and will be held 3-4 times per year.

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